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TRANSACTIONS
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COMMUNICATIONS.*

THE SUSCEPTIBILITY OF THE SOUTH AFRICAN GERBILS (GENUS *TATERA*) TO RICKETTSIAL DISEASES AND THEIR USE IN THE PREPARATION OF ANTI-TYPHUS VACCINE.

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In the course of an investigation to determine the susceptibility of various veld rodents to tick-bite fever, it was discovered that both the Transvaal gerbil (*Tatera brantsi*) and the Cape gerbil (*T. afra*) are very susceptible, and often die of the infection. As this discovery appeared to us to be of considerable interest a more detailed study was undertaken, and the susceptibility of gerbils to epidemic louse-borne typhus and endemic flea-borne typhus was also tested.

The results were found to be of practical significance as they indicated that the gerbil is a suitable animal for use in obtaining large amounts of the rickettsiae of louse typhus, and of tick-bite fever, as well as of flea typhus. The yields are so large that there is no doubt that gerbils can be used to prepare vaccines from *Rickettsia prowazeki*, the causative organism of louse typhus, and from *R. rickettsii* var. *pijperi* the causative organism of tick-bite fever, in the same way

* Owing to difficulties created by the war, meetings at which Papers are read are not being held at present. In consequence these TRANSACTIONS commence with Communications instead of with a Paper as has been the custom in normal times.

that white rats are used to prepare vaccine from *R. mooseri*, the causative organism of flea typhus, by the Zinsser-Castaneda method. This is shown in the following experiments :—

A.—EXPERIMENTS WITH TICK-BITE FEVER.

Experiment 1.—Eight gerbils (*T. afra*), which had been collected alive in Citrusdale, Cape Province, and kept in captivity for several months by one of us (D. H. S. D.) were each injected intraperitoneally with 2 c.c. of infective peritoneal washing obtained by rinsing in 20 c.c. of normal saline the testes removed from a guineapig at the height of an attack of tick-bite fever. Of these eight gerbils two died on the 4th day and were eaten by their comrades, so that it was not possible to examine peritoneal smears for the presence of rickettsiae.

Three died on the 5th and 6th days after inoculation. All showed numerous rickettsiae in the peritoneal smears, but one showed very large numbers comparable to the growth of *R. mooseri* obtained in the peritoneal cavity of a white rat inoculated after exposure to X-rays.

Two died on the 7th day after inoculation and also showed numerous rickettsiae but not in such profusion.

One recovered, although for 4 days it was obviously ill, having puffed-up eyes and a staring coat.

Experiment 2.—Six gerbils (*Tatera brantsi*) from amongst a number collected by Mr. SMITH, Rodent Inspector of the Public Health Department of the Johannesburg Municipality, were inoculated intraperitoneally with the peritoneal washings from an infected guineapig taken at the height of the fever as before. Two died and were eaten by their companions before the 4th day.

One died on the 6th day after inoculation and impression smears made from the peritoneal surface of the liver and spleen showed numerous rickettsiae.

Two recovered after an obvious illness lasting approximately one week.

As it was apparent that although a heavy infection often occurred, this was not invariable, it was considered that exposure to X-rays might reduce the resistance so that all or nearly all gerbils would show a profuse growth of rickettsiae. This was found to be so in the following experiment :—

Experiment 3.—Five gerbils from the same batch as those used in the previous experiment were exposed to the action of X-rays by Miss MILLAR, of the X-Ray Department of the Johannesburg Hospital. An hour later they were inoculated with a heavy suspension of rickettsiae obtained from the yolk sac of a developing chick embryo infected by inoculation with a strain of tick-bite fever. The yolk sac was ground up in a tube with powdered glass and 20 c.c. of normal saline were added. Each gerbil received 2 c.c. of this emulsion intraperitoneally. One gerbil died on the 2nd day and was eaten.

Three of the other four died on the 5th day and the remaining one on the 7th day. All showed very numerous rickettsiae in smears made from the peritoneum, again comparable in profusion to the growth of *R. mooseri* in the peritoneal cavity of X-rayed white rats, several hundred rickettsiae being seen in each microscopic field.

OBSERVATIONS ON THE MORPHOLOGY OF THE RICKETTSIAE SEEN IN THE SMEARS.

Several interesting observations were made on the morphology and cytoplasmic distribution of the rickettsiae. Apart from the very numerous organisms lying free in smears made from the peritoneum, *e.g.*, from the surface of the spleen, in general the distribution of the rickettsiae corresponded closely to the description given for guineapigs, except that the infection was very heavy. Numerous mononuclear cells were seen containing scattered rickettsiae in the cytoplasm which showed vacuolation. However, unlike the distribution in the

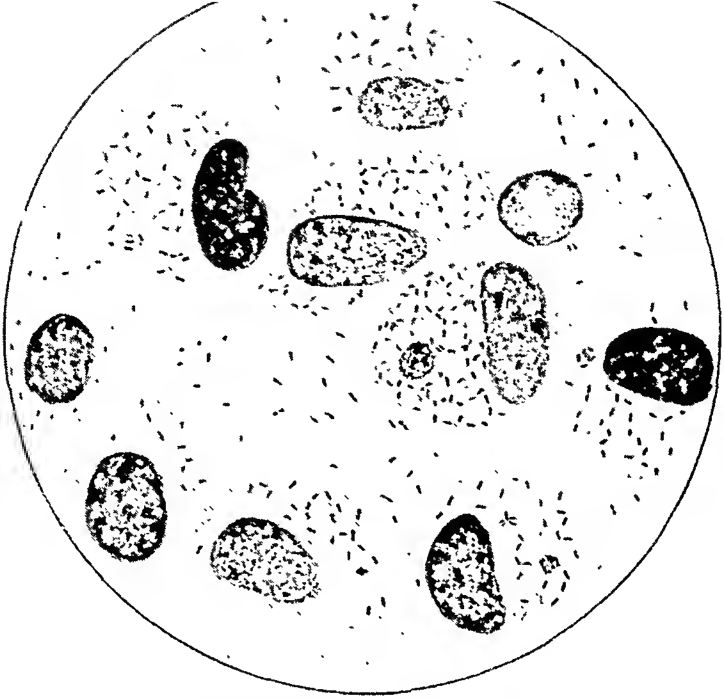

FIG. 1.—*Rickettsia rickettsii* var. *pijperi*, the rickettsia causing tick-bite fever. From a painting of a smear made from the peritoneal surface of the spleen of a gerbil, *Tatera brantsi*, infected with tick-bite fever.

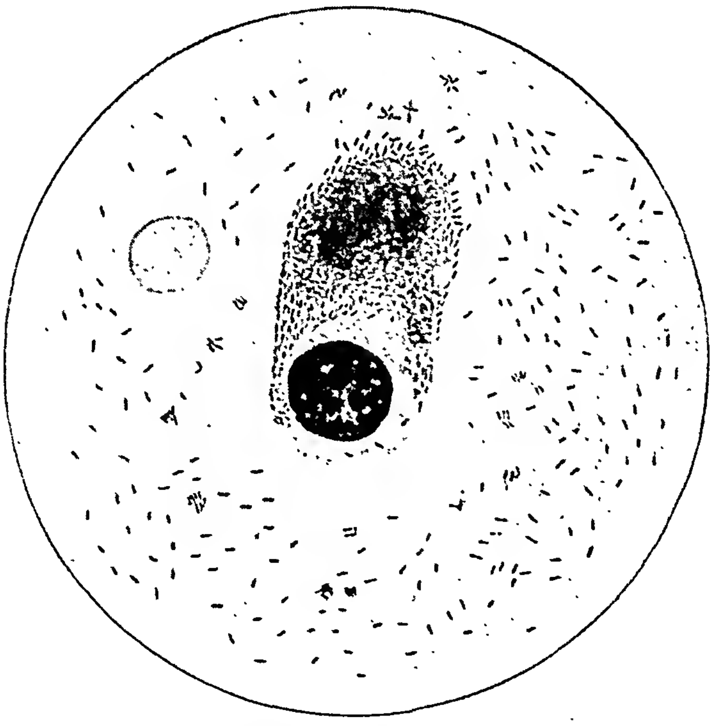

FIG. 2.—*Rickettsia prowazeki* the rickettsia causing epidemic typhus. From a painting of a smear made from the peritoneal surface of the spleen of a gerbil, *Tatera brantsi*, infected with epidemic louse-borne typhus.

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guineapig, many of the cells showed rickettsiae apparently within the nucleus although it was naturally difficult to be certain that the rickettsiae were not superimposed on the nuclei. Against this, however, was the fact that often there was a clear halo in the nucleoplasm surrounding the rickettsiae. The apparently intranuclear organisms were especially clearly evident when the smears were stained by Pinkerton's method.

Another rather unusual finding, not yet described in tick-bite fever, was that often small circular masses of rickettsiae, somewhat resembling the appearance of rickettsiae of heartwater, were seen in the cytoplasm of the cells.

As the gerbil is the only animal so far discovered to suffer from a heavy and often fatal infection of the rickettsiae causing tick-bite fever, it was considered possible that an equally profuse growth of the rickettsiae causing louse typhus and flea typhus might occur. This was found to be the case as is indicated in the following experiments :—

B. Experiments with Louse Typhus.

Experiment 1.—Five gerbils (*Tatera brantsi*) were inoculated intraperitoneally with 2 c.c. of an infective suspension prepared by emulsifying in 20 c.c. of normal saline the brain removed from a guineapig at the height of an attack of louse typhus, *i.e.*, on the 10th day after infection.

The next day one gerbil was found dead but no rickettsiae were observed in smears made from the surface of the liver and spleen.

On the 5th day one was killed and moderate numbers of rickettsiae were seen in the smears from the peritoneum.

Two died after the 12th day but were not examined for rickettsiae.

One recovered.

Experiment 2.—Six *Tatera afra* that had been kept in captivity for several months were inoculated intraperitoneally with the peritoneal washings and brain emulsion from the gerbil killed in the previous experiment.

Four days later two died and numerous organisms of pleuro-pneumonia were seen in the smears made from the surface of the liver and spleen.

On the 5th day another gerbil was found dead but not examined.

On the 7th day yet another gerbil was found dead and numerous rickettsiae as well as organisms of pleuro-pneumonia were seen.

The remaining two were discarded.

This experiment indicated that the gerbil used as a source of the infective material was infected with pleuro-pneumonia as well as louse typhus. As this infection must have been contracted by contact with infected mice kept in the same animal room, arrangements were made for the stringent isolation of all recently caught gerbils arriving at this Institute. The following experiments were conducted with these isolated animals. None of them showed signs of pleuro-pneumonia, the commonest of which appears to be acute arthritis with marked swelling of the affected joint.

Experiment 3.—Six gerbils (*Tatera brantsi*), after exposure to X-rays, as in previous experiments with tick-bite fever, were inoculated each with 2 c.c. of brain emulsion prepared by emulsifying in 20 c.c. of normal saline the brain of an infected guineapig killed at the height of fever on the 10th day after inoculation with louse typhus.

Three days later one gerbil was found dead and partially eaten.

Five days later two were found moribund and were killed. A prolific growth of rickettsiae was seen in smears made from the peritoneal fluid.

On the 6th day two died and again large numbers of rickettsiae were seen in peritoneal smears.

On the 7th day the remaining one died and again large numbers of rickettsiae were seen in the peritoneal smears.

The peritoneal cavities of these five gerbils were scraped and washed out with 0·4 per cent. formol saline, and it was found possible to obtain 5 to 20 c.c. of a suspension of rickettsiae of a density approximately equal to 1,000 million *B. coli* per c.c. from each gerbil.

Finally the susceptibility of the gerbil (*Tatera brantsi*) to murine typhus was determined.

C.—Experiment with Murine Flea Typhus

Four gerbils, after exposure to X-rays as before, were inoculated intraperitoneally with a suspension of rickettsiae obtained from the peritoneal cavity of an infected white rat.

These gerbils were found moribund on the 5th day after inoculation and were killed by chloroform.

Smears made from the peritoneum in each case showed an extraordinarily heavy infection with rickettsiae—heavier than has yet been seen in X-rayed rats—giving in many places the appearance of a confluent growth.

By washing out the peritoneal cavities of these gerbils with 0·4 per cent. formol saline, 10 to 20 c.c. of a suspension of rickettsiae of a density approximately equal to 1,000 million *B. coli* was obtained from each animal.

Discussion

These experiments have proved that the two common gerbils of South Africa, *Tatera brantsi* and *Tatera afra*, are susceptible to the three forms of typhus occurring in this country, namely epidemic louse-borne typhus, endemic murine or flea-borne typhus and tick-bite fever, the South African variety of tick typhus. Their susceptibility to epidemic typhus and tick bite fever is particularly noteworthy.

After exposure to the action of X-rays, the dose given being 600 R, using a 200 K.V. and 8 M.A., with a F.S.D. of 40 cm., and a 0·5 cm. copper filter, the resistance of the gerbils is lowered so that after intraperitoneal inoculation almost every animal dies of the infection with an extremely heavy rickettsial growth in the peritoneum. In profuseness this rickettsial growth is comparable to that seen when white rats are inoculated with murine typhus after exposure to X-rays, as in the Zinsser-Castaneda method for preparing vaccine from *Rickettsia mooseri*.

Unfortunately, Zinsser and Castaneda found that similar profuse growths of *R. prowazeki* did not occur in white rats, even after these animals had been exposed to X-rays. Similarly, Mr. C. Bevan, working at this Institute, has found that although there is an increase in the number, the rickettsiae of tick-bite fever also do not grow prolifically in white rats inoculated after exposure to X-rays.

It is apparent then that white rats cannot be used for obtaining large amounts of the rickettsiae of epidemic typhus or of tick-bite fever for the preparation of vaccines, nor up to the present had a laboratory animal suitable for this been discovered. However, the experiments detailed above clearly indicate that the

common gerbils can be used for this purpose. This has already been done and from 5 to 20 c.c. of a formolized suspension of rickettsiae, suitable for use as a vaccine, has been obtained from each gerbil inoculated, and experiments are already under way for testing the effectiveness of this vaccine.

Of course, the practicability of using gerbils for large-scale production of anti-typhus vaccines depends on the number of gerbils available, the ease with which they can be collected and kept in captivity and the ease with which they can be handled in the laboratory. At present many local authorities spend considerable efforts in attempts to exterminate these rodents, which are well known as the principal rodent reservoir of plague in South Africa. By using them for the preparation of anti-typhus vaccines a double purpose is achieved, large numbers of gerbils will be eradicated, and at the same time put to an extremely useful purpose.

As laboratory animals gerbils are hardy, keeping in good condition on a diet of comproid mouse biscuit, carrot, cabbage and water, are very easily handled and submit to laboratory manipulation more readily than white rats and white mice. They do not breed readily enough in captivity to warrant the establishment of a breeding stock, so that the supply must be wild caught. It remains for us to indicate briefly, by describing the salient features of their natural history, that an adequate supply could be maintained, provided facilities for their collection were available.

Gerbils of the genus *Tatera* are widely distributed throughout the African continent and extend as far as India through Arabia and Asia Minor. They are burrowing rodents characteristic of desert, steppe and open bush country and are found chiefly in sandy soils. In South Africa the four commonest species are: *Tatera lobengulae* (N. and W. Transvaal and N.W. Orange Free State), *T. brantsi* (Orange Free State and S. and E. Transvaal), *T. schinzi* (South-West Africa) and *T. afra* (Cape Midlands, S. and S.W. Cape). They differ in colour and size but little in habits (ROBERTS*). They are colonial, nocturnal animals and are never anywhere but in their warrens during the day. They live in burrows which are usually not more than 1 foot but may be 3 or more feet deep, according to the soil. A colony is made up of anything from a few warrens (about twenty burrows interconnected to form an average warren) to several hundred. Soil and food supply control the extent of colonization. Where the soil is light and easily burrowed into and where their favourite food (the corms of the sedge, *Cyperus esculentus*) is in abundance, they may reach a population density of from 50 to 350 individuals per morgen ($2\frac{1}{4}$ acres) and a colony strength of between 500 and 1,000. It is more usual to find them in smaller colonies of between 10 and 50 animals with a population density of from 5 to 15 per morgen. As a rule the finding of a gerbil colony means that there

*ROBERTS, A. (1935). Mammals concerned in the bubonic plague and rabies problems in South Africa. *S. Afr. J. Sci.*, 33, 414.

is another within half a mile or so. It is rare not to find a colony on an average sized farm of 1,000 morgen if soil and food conditions are suitable.

Over large parts of the Union they form the enzootic reservoir of plague and as a consequence never reach the high population densities found in plague-free areas. The two most important plague-free gerbil infested areas are the Eastern and Northern Transvaal highveld and the sandy coastal plains in the Cape Province to the North and East of Cape Town. Although some country districts of the Transvaal are heavily infested it is not necessary to go so far afield. At one point a few miles south of Johannesburg there is at the present time a particularly rich concentration of gerbils. From recent surveys it has been estimated that some 2,000 animals could be obtained from this area, and this and similar areas at other points along the Reef will serve as a convenient pool from which to draw supplies for the laboratory.

However, to obtain a sufficient number of gerbils for large-scale vaccine production will take the full time of a rodent gang. Fortunately a number of such gangs are already operating in the service of several municipalities of the Witwatersrand, in addition to those working in the service of the Union Health Department. There will be no difficulty in obtaining adequate supplies from these gangs without interfering with their regular work, a large part of which is already devoted to the destruction of gerbils. It is proposed that a central depot should be established, functioning under the control of the Chief Rodent Officer of the Union Health Department to which gerbils will be sent and where they will be deverminized and cared for until ready for use.

Summary.

During experiments to determine the susceptibility of various veld rodents to tick-bite fever, the variety of tick typhus occurring in South Africa, it was discovered that the South African gerbils (*Tatera brantsi* and *T. afra*) are very susceptible and often die showing a heavy infection of the peritoneum.

After exposure to X-rays to lower their resistance most animals showed a very heavy infection of the peritoneum comparable to the growth of rickettsiae occurring in X-rayed white rats inoculated with a murine strain of typhus.

Further experiments showed that these gerbils were also susceptible to epidemic louse-borne typhus and after exposure to X-rays the growth of *Rickettsia prowazeki* is also as profuse as the growth of *R. mooseri* in X-rayed white rats.

Finally, it was shown that the inoculation intraperitoneally of *Tatera brantsi* with a murine strain of typhus, after exposure to X-rays, yielded a growth of rickettsiae more profuse than that seen in white rats similarly inoculated.

These findings indicate clearly that the gerbil is suitable not only for the preparation of vaccine from murine typhus strains, but also for the preparation in a similar way of vaccines from strains of epidemic typhus and tick-bite fever. As far as is known the gerbil is the first animal to be discovered suitable for this purpose.

It will be possible to obtain adequate supplies of gerbils for large-scale vaccine production from the several rodent gangs operating in the Union.

Acknowledgments.

We are indebted to Sir EDWARD THORNTON, Director General of Medical Services, and Dr. PETER ALLAN, Secretary for Public Health, for permission to publish an account of this work, and to Dr. E. H. CLUVER, Director and Dr. G. BUCHANAN, Deputy Director, of the South African Institute for Medical Research, for their interest in its progress.

We also wish to record our grateful thanks to Mr. SMITH, Rodent Inspector of the Public Health Department of the Johannesburg Municipality, who, with the permission of Dr. LAING, Chief Medical Officer of Health, kept us adequately supplied with gerbils during the investigation; and to Dr. MURRAY CRAIB, Senior Radiologist to the Johannesburg Hospital, for his advice and his permission to allow Miss MILLAR to X-ray gerbils and white rats. We are very grateful to Miss MILLAR for so willingly devoting much of her time to this work.

We also wish to acknowledge the assistance with the experiments of Mr. C. BEVAN, Technician at the South African Institute for Medical Research, and of S./Sgts. HARRIS, SANER and OSBURN, Technicians in the laboratory service of the South African Medical Corps.

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YELLOW FEVER IN WESTERN UGANDA.

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The results of the yellow fever immunity survey of Central Africa reported by SAWYER and WHITMAN (1936) revealed a much wider distribution of immunity than could have been anticipated from the history of the disease. Apart from a single case (HEWER, 1934) yellow fever had never been recognized in Central Africa. The survey, however, demonstrated that many persons in the south-western part of the Anglo-Egyptian Sudan and a smaller number near the northern and western borders of Uganda had acquired immunity. The almost complete absence of reported cases in this area led some persons to doubt the validity of the mouse protection test while others believed that if, as indicated, the disease had been widespread over this vast area it must have occurred in an unrecognizable form. With the opening of this Institute late in 1936 intensive studies were begun which it was hoped might provide the answers to some of the questions raised by the results of the immunity survey. The investigation was initiated in Uganda and later extended to include the southern Sudan and portions of the Belgian Congo. While this work was in progress yellow fever appeared in epidemic form in the Nuba Mountains district of the Anglo-Egyptian Sudan (KIRK, 1941) and two strains of virus were isolated from cases

* A considerable number of persons have contributed to and assisted with this programme of work. For their co-operation and assistance we wish to express our appreciation and thanks to the officers of the Medical Department of the Uganda Government; to the administrative officers of the Western Province; to Mr. J. O. HARPER and Mr. E. G. GIBBINS who carried out entomological studies and acted as field assistants; to Dr. J. H. PAUL who did the early protection tests and to Dr. T. P. HUGHES who assisted with the collection of blood specimens and did many of the protection tests. We are especially indebted to Dr. J. C. ST. GEORGE EARL, Senior Medical Officer, Western Province, whose early suggestions and interest and whose continued enthusiastic collaboration did much to further the investigation.

† This Institute is supported jointly by the Medical Department of the Uganda Protectorate and the International Health Division of The Rockefeller Foundation.

seen there (Mahaffy, Hughes, Smithburn and Kirk, 1941). In the meantime the studies in Uganda, with which this report is concerned, have revealed an important focus of the disease in that part of the valley of the Semliki river which lies west of the Ruwenzori Mountains and which embraces the most easterly reaches of the Ituri forest (Bwamba County). A recent outbreak of yellow fever occurred in this area during the course of which virus was isolated from a case in a human and from wild-caught mosquitoes.

Our attention was first directed to Bwamba County by Dr. J. C. Earl, Senior Medical Officer, Western Province, who suggested it as the most promising place in which to search for yellow fever in Uganda. The area adjoins the Belgian Congo and lies in the valley of the Semliki river west of the Ruwenzori Mountains. The northern half of the county is heavily forested and uninhabited except for a few pigmies, while the southern sector has an estimated population of 25,000 Africans. There are no Europeans resident in the area but it is visited from time to time by government officials in the course of their duties. The Medical Department maintains a dispensary at Bundibugyo.

During a visit to the dispensary at Bundibugyo early in 1937, Dr. Earl collected blood samples from fifty-four adult residents and when these were examined in the protection test twenty-five of them gave a positive result. This was a much higher percentage of immunes than had been found in other places in Uganda, and it indicated that yellow fever had been prevalent in Bwamba in the recent past. At this time Bwamba was a relatively inaccessible area which could only be reached on foot by a path leading over the mountain. Work had, however, been commenced on a road which ultimately would join provincial headquarters at Fort Portal with county headquarters at Bundibugyo. After passing over the north end of the mountain the projected road entered Bwamba County and passed through several miles of uninhabited forest before bearing left to end at Bundibugyo. Labour was recruited for the most part in Bwamba and in the adjoining district in the Belgian Congo. Construction reached the edge of the heavy forest late in 1937, at which time a field station was established near the labour camp and the workmen were kept under close observation for evidence of yellow fever. The field station moved with the labour camp and the observations were continued until the road was completed towards the end of 1938. The number of labourers employed varied between 400 and 900 and there was a very heavy turnover at the end of each month. The total number of persons who came under observation was therefore very much higher than the number at any one time. Individuals who exhibited febrile illness without obvious cause were bled and their sera were inoculated into white mice. Inoculated animals were kept under observation for a period of 1 month. A new clinical entity was studied and its causative virus was isolated (Smithburn, Mahaffy and Paul, 1941) but the presence of yellow fever was not detected.

After the completion of the road, field headquarters were established at the

government dispensary at Bundibugyo and the resident population was kept under observation almost without interruption until September, 1939. During this time clinical yellow fever was not recognized and all attempts to isolate virus failed.

In addition to the search for cases and the attempts to isolate virus entomological surveys were made both in the uninhabited forest and in the populated areas. Unsuccessful efforts were made to isolate virus or to demonstrate its presence by the inoculation of wild-caught mosquitoes into rhesus monkeys. A viscerotomy service, commenced in December, 1937, was continued for nearly 2 years during which time 127 liver specimens from deceased residents failed to show lesions of yellow fever. Although none of this work produced evidence that the virus was actually present in the area at the time, the results of large numbers of protection tests continued to demonstrate its recent presence, since young children were found to be immune.

At the outbreak of the war the permanent field station was closed. Contact was, however, maintained with the area through extended protection test surveys made in October, 1939, and during the early months of 1940. The results of these and earlier tests, which have already been published (HUGHES, JACOBS and BURKE, 1941), showed that immunity is not evenly distributed throughout the area. The percentage of immune individuals of all age groups in the southern grassland portion of the county was about 8 per cent. as compared with 20 per cent. in the northern section where there is more forest. Furthermore, the highest percentage of immune individuals was found in villages situated along the edge of the unhabited heavy forest on a line joining Hakitengya with Butoga. However, no locality was found where the inhabitants had been immunized to a degree which would correspond to that following epidemics of urban yellow fever. Since the infection was known to have been present recently it was believed that the disease in Bwamba was endemic, or, if epidemic, that for some reason the outbreaks did not immunize the mass of the population.

In April, 1940, two small areas in the more highly immunized zone between Hakitengya and Butoga were selected for concentrated study. An experienced worker was placed in charge of each area and the entire population, amounting to about 600, was visited daily for a period of 1 month. All persons found with an elevated temperature, the cause of which was not obvious, were bled and their sera inoculated into white mice. This work did not reveal any illness clinically resembling yellow fever nor was the virus of that disease isolated.

In view of the fact that all our attempts to find cases of yellow fever in Bwamba had thus far failed we next attempted to demonstrate the recent presence of the disease by periodic examination of the sera of selected non-immune donors. For this purpose a group of 275 individuals who had been shown to be nonimmune in October, 1939, or in January, 1940, were chosen. Ninety-seven of these were bled again in April and an additional seventy-one

in June, 1941. When these specimens were tested it was found that forty-eight of them gave a positive result. Identification of donors was established when the thumbprints taken at the time of both the first and second bleedings were examined by an expert from the Uganda Police Department and declared to be identical.* Thus it became evident that forty-eight of these 168 Bwamba residents had become immune against yellow fever between October, 1939, and June, 1941, and twenty-six of these had become immune by April, 1941.

It is perhaps noteworthy that ninety-seven of the original group of selected donors could not be found and relatives of some of these persons stated that they had died. However, careful interrogation of native chiefs and other persons in the community did not elicit any reliable evidence that a fatal illness with symptoms characteristic of yellow fever had occurred in the area. Many of those who had recently become immune admitted having had febrile illness during the past year but they were unable or unwilling to describe the symptoms. It was our impression that the majority of the cases of yellow fever were probably mild and that the number of severe and fatal cases occurring at any one time was not sufficient to attract special attention.

When it became known that the disease had actually been present in Bwamba within the last 18 months an investigation was at once undertaken to determine whether the virus was still active. To prevent possible eastward spread of the disease the Medical Department of the Uganda Government instituted a programme of mass vaccination in the Toro District east of Bwamba and an immune zone was quickly created around the area of recent infection. Bwamba County was later included in this programme but, before immunization could be commenced there, the presence of the disease had been conclusively demonstrated.

The investigation, which included clinical, epidemiological, and entomological studies, was concentrated in the six local districts where residents were known to have been recently immunized. A viscerotomy service was again established and every effort was made to secure liver specimens from all deceased persons whether suspect or not. African assistants trained in the use of the thermometer were sent out to search for cases of febrile illness and, when found, these were investigated. If they were seen during the early stage of the illness and if the symptomatology was suggestive they were bled and the sera inoculated into mice. Less suspicious cases were bled early in the illness and again after recovery and both specimens were examined in the mouse protection test. During the course of this brief study two cases were seen in which a clinical diagnosis of yellow fever seemed justified, and a strain of virus was isolated from one of them.

Entomological studies (by J. D. G.) quickly revealed that *Aëdes (Stegomyia) simpsoni* Theobald was the only species of mosquito which could be captured

* Seven specimens which gave a positive result in the second test were excluded because the thumbprints were either not identical or were unsatisfactory for comparison.

in daytime in large numbers. It breeds chiefly in plant axils and the adults were most numerous in the cultivated areas around dwellings. It feeds readily on man during daylight and is more active in bright than in dull weather. As this species has been shown to be a potential vector of yellow fever (Philip, 1929) attempts were at once made to isolate virus from wild-caught specimens. Capture squads were organized and, using themselves as bait, were set to work near houses where suspect cases were under observation or had recently occurred. The captured mosquitoes were identified with a hand lens and sent to the laboratory in ant-proof Barraud cages. As the Bwamba road was closed at this time it was necessary to have the cages carried over the mountain by porters but they usually reached the laboratory about 48 hours after capture and there were relatively few deaths.

Mosquitoes received at the laboratory were killed with chloroform and ground in a mortar with 10 per cent. of normal human serum in physiological saline. The suspensions were centrifuged and mice were inoculated with the supernatants. The latter were then passed through Seitz EK pads under 15 pounds air pressure and the filtrates were inoculated into additional groups of mice and into normal rhesus monkeys. Mice which became ill were sacrificed for brain passage and for this purpose 10 per cent. suspensions of brain were prepared in 10 per cent. serum-saline.

The temperatures of the monkeys were taken twice daily. When an animal registered 104° F., or higher, it was bled and the serum inoculated into normal mice. Animals which died were examined postmortem and the tissues were studied microscopically. One which died without exhibiting fever and which showed characteristic lesions of yellow fever was tested for virus by subinoculation of liver suspension intracerebrally into mice and subcutaneously into another normal rhesus monkey.

Mice inoculated in the field with serum from suspect cases were observed for 30 days. Animals which showed signs of illness were sacrificed and brain passage was made to normal mice. In two instances the brain emulsions from sick mice were inoculated subcutaneously into normal rhesus monkeys.

History of Three Strains of Yellow Fever Virus Isolated in Bwamba.

Strain 1.—Isolated from a Patient during an Attack of Yellow Fever

An African female, age 27 years, seen on 23rd June, 1941, complained of severe headache and pain in the back of the neck. Her temperature was 104·6° F. and pulse 120. She stated that she became ill in the evening of the previous day. The patient looked ill but physical examination revealed no outstanding signs. There was moderate conjunctival injection and the tongue was small and pointed, with red tip and edges, and with a moderate coat over the dorsum. The blood smear showed no parasites and the urine contained no albumin. The patient was bled at 1 p.m., 23rd June, and the serum inoculated intracerebrally into a group of six white mice 1 hour later.

The patient was seen again on 25th June when the temperature was 103·6° F., pulse 118. She complained of very severe frontal headache and pain in the back of the neck. Prostration was marked. Faint icterus of the sclerae was noted. The urine showed a

light cloud of albumin. On 27th June the temperature was 103·4° F., pulse 112. She complained of persistent severe headache, pain in the neck and, for the first time, of pain in the back and in the epigastrium. There was marked epigastric tenderness and the sclerae were now lemon yellow in colour. The following day the complaints were unchanged, temperature 103·4° F., pulse 118. The urine showed a heavy deposit of albumin. The eyes showed definite jaundice. She stated that she had passed no urine the previous day but this was doubted. There was no vomiting. Her condition showed slight improvement during the next 2 days but on 1st July she developed a severe respiratory infection with productive cough but without definite chest signs. The patient made a slow but apparently complete recovery and was quite well on 16th July, when she was bled for an immunity test. The first specimen of serum gave a toxic reaction in the yellow fever protection test and it was probably contaminated when opened for inoculation of mice. The second specimen gave a positive result.

The six mice inoculated with the patient's serum on 23rd June remained well for 17 days. On the 18th day one mouse was dead, two were sick, and one was hyperactive. The three abnormal animals were sacrificed, and separate 10 per cent. suspensions of the three brains were prepared in 10 per cent. of normal human serum in physiological saline (hereafter called serum-saline diluent). The suspensions were lightly centrifuged and inoculated intracerebrally into three groups of ten mice each. The remaining suspensions were then pooled and normal rhesus Monkey 192 was inoculated subcutaneously with 5·6 c.c. of the pool.

The two remaining mice of the original group remained well and were discarded 30 days after inoculation. The three groups of second-passage mice also remained well for 5 weeks when they were discarded. Monkey 192 did not at any time have a temperature in excess of 103·2° F. and did not appear ill, but was found dead on the morning of the 22nd day after inoculation. Autopsy showed cutaneous congestion and icterus; the liver was normal in size, pale yellow-brown (boxwood) in colour, and appeared slightly greasy in section; the stomach contained a large quantity of altered blood and some greenish-black fluid. Sections of the liver exhibited typical microscopic lesions of yellow fever.

A portion of the liver of Rhesus 192 was used to make a 10 per cent. suspension in serum-saline diluent and mice were inoculated intracerebrally with unfiltered and filtered portions. Mice receiving the unfiltered suspension became ill on the 5th and 6th days and were all dead by the 12th day after inoculation. Those receiving the liver filtrate were ill on the 6th day. Eight sick mice were sacrificed and used for preserving the virus by drying and the remaining four all died on or before the 9th day.

Subinoculations in series have been made from mice of the group inoculated with unfiltered liver suspension from Rhesus 192. The incubation period was 5 days during the first five passages but decreased to 4 days in the sixth passage. The transmissible agent was shown to be neutralized by yellow fever immune serum but not by serum from known nonimmune persons.

A suspension of the brains of sick mice from the group inoculated with the liver emulsion of Rhesus 192 was injected subcutaneously into Rhesus 185. This animal showed no fever but had a subnormal temperature (98·2° F.) on the morning of the 3rd day and appeared very ill. It was found dead on the morning of the 4th day and the gross and microscopic lesions were typical of yellow fever. Monkey 185 was bled on the 3rd day and the serum had a virus titre in mice of 1 to 23,000,000 by the method of Reed and Muench (1938). A portion of this serum was preserved by drying while frozen and 1·8 c.c. of the remainder was inoculated subcutaneously into normal Rhesus 181.

Rhesus 181 had a fever of 104·4° F. 30 hours after inoculation and elevated temperature daily thereafter through the 5th day. It first appeared ill on the 4th day. The temperature was subnormal (92° F.) on the 7th day and the animal was moribund. It was sacrificed and found to have typical gross and microscopic lesions of yellow fever.

It will be noted that this strain of virus failed to become established by continuous passage in mice but readily did so after passage through one rhesus monkey. In all its pathogenic and immunologic properties it behaves like other strains of yellow fever virus isolated in this and other laboratories.

STRAIN 2.—ISOLATED FROM WILD-CAUGHT MOSQUITOES.

Two Barraud cages containing 408 (lot 8) and 500 (lot 9) adult females of *A. simpsoni* caught at Bundimbale, Bubukwanga and Bundiwerume were received at the laboratory on the evening of 7th July. Each cage contained a few dead mosquitoes but the majority were alive and active. The following morning they were killed with chloroform and each lot was ground separately in a mortar with serum-saline diluent to make a suspension of approximately 10 per cent. strength. The suspensions were centrifuged for half an hour at about 3,000 r.p.m. and groups of mice were inoculated with each supernatant. The remaining supernatants were passed through Seitz E K pads and ten mice were inoculated with each filtrate. The remainder of the two filtrates was then pooled and 9·0 c.c. of the pool were inoculated subcutaneously into normal rhesus Monkey 198.

Each of the unfiltered mosquito suspensions caused the death of inoculated mice within 24 hours. The mice inoculated with the filtrate of lot 8 remained well during 30 days. One of ten mice receiving lot 9 filtrate was dead on the 15th day without previously having appeared ill; the remaining nine mice remained well for 30 days when they were discarded. There were no passages from the original groups.

Monkey 198 had no temperature in excess of 103·4° F. during 29 days. The morning temperature on the 30th day was 103·8° F. and the animal was bled and the serum inoculated into a group of mice. The temperature was normal on the following day but the animal looked ill. On the morning of the 32nd day it was moribund and the temperature had dropped to less than 92° F. With difficulty enough blood was obtained for the inoculation of a group of ten mice. The animal was sacrificed and postmortem studies revealed characteristic gross and microscopic lesions of yellow fever.

A portion of the liver of Rhesus 198 was removed aseptically and a 10 per cent. suspension was prepared in serum-saline diluent. The suspension was centrifuged lightly and portion of the supernatant was used to inoculate a group of mice. The remainder was filtered through a Seitz E K pad and the filtrate was injected intracerebrally into a group of mice and subcutaneously (3 c.c.) into normal Rhesus 196.

All the mice inoculated with 30th day serum of Rhesus 198 became sick on the 5th day and all were dead on the 10th day. One sick mouse of this group was sacrificed for passage and by successive passages a line of virus was established. Mice inoculated with serum taken from Rhesus 198 when it was moribund behaved in identical fashion and a second line of virus was established by passage in the 5th day. Mice receiving the unfiltered and filtered liver suspension became ill on the 5th and 6th days respectively and all animals of both groups were dead by the 10th day. A third line of this strain of virus was established by passage on the 5th day from mice receiving unfiltered liver suspension.

Rhesus 196, which was inoculated with the filtrate of liver suspension from Rhesus 198, had fever on the 3rd, 4th, 5th, 6th, and 9th days, and appeared ill. The animal recovered and serum taken 20 days after inoculation contained abundant neutralizing antibody against standard neurotropic yellow fever virus.

Two of the three lines of this strain of virus have been transmitted in series in mice. In one line the incubation period was 5 days through three passages, and 4 days thereafter. In the other line it was 5 days through five passages, and 4 days in the sixth and succeeding passages.

Virus of the third mouse passage was tested against known nonimmune and yellow fever immune human sera in the intraperitoneal protection test. The normal serum gave no protection while the yellow fever immune serum protected all the test animals.

This strain of virus, as did the previous one, failed to become established by direct passage in mice but it was readily transmissible in mice after one passage through a rhesus monkey.

It is perhaps noteworthy that some of the mosquitoes of the two lots giving rise to this strain of virus were caught in the immediate vicinity of the house occupied by the patient from whom Strain I was isolated.

STRAIN 3.—ISOLATED FROM WILD-CAUGHT MOSQUITOES.

Two Barraud cages containing 345 (lot 10) and 305 (lot 11) female *A. simpsoni* caught at Bubomboli were received at the laboratory in the evening of 14th July. Next morning

they were killed with chloroform and treated in precisely the same manner as in the case of lots 8 and 9. Mice were inoculated intracerebrally with unfiltered and filtered suspensions of each lot and 6·6 c.c. of the pooled filtrates were injected subcutaneously into normal Rhesus 188.

All the mice inoculated with these suspensions remained well, except one which developed hydrocephalus, and there were no passages from the original group.

Rhesus 188 had no significant elevation of temperature until the afternoon of the 27th day when it rose to 105·2° F. The animal was bled and mice were inoculated with the serum. The temperature was still above normal (104·6° F.) the next morning and it was again bled and mice inoculated with the serum. Ten ampoules of this serum were dried while frozen and another portion was used as virus in the intraperitoneal mouse protection test. The monkey was moribund the following day and was sacrificed. The gross and microscopic lesions were those of yellow fever.

Mice inoculated with the 27th day serum of Rhesus 188 were ill 5 days after inoculation and two lines of this strain of virus were established by passage from this group. All the remaining mice were dead by the 9th day. Mice inoculated with serum taken on the 28th day also became ill on the 5th day and all were dead by the 9th day.

Serum of Rhesus 188 taken on the 28th day was used as virus in the mouse protection test with known normal and yellow fever immune sera. The animals inoculated with the normal serum-virus mixture all died and those receiving the immune serum and virus were fully protected. This result provided proof that the virus circulating in the blood of Rhesus 188, and subsequently established by serial passage in mice, was yellow fever virus.

The two lots of mosquitoes from which this strain of virus was isolated were caught in a locality where twenty-one of thirty-five persons were found by the protection test to have recently become immune to yellow fever. Because of the high proportion of immune individuals no concentrated effort was made to find cases of the disease in the Bubomboli area. However, the fact that infected mosquitoes were found there suggests that the disease was still present or that cases had very recently occurred.

COMMENT.

The geographical area included in these studies may be broadly indicated as the basin of the Lamia river, a tributary of the Semliki river. This area lies just north of the Equator at an altitude of about 2,500 feet above sea level and includes not only Bwamba County in Uganda but also the adjacent country in the Belgian Congo known as the Watalinga District. Certain characteristics of the topography, climate, flora, fauna, and people of these two portions of the Lamia basin demand that they be considered as one, but from the standpoint of the epidemiology of yellow fever there are certain important differences which will be mentioned later.

The Lamia basin is in reality a portion of the great rift valley which begins with Lake George at its upper, southern end, and includes the Kazinga Channel, Lake Edward, the Semliki river, Lake Albert and the Albert Nile far to the north. This valley, at least in its lower reaches, was a huge lake in ancient times, evidence of which are the lake-bottom sedimentations known as the Kaiso beds of to-day. It diverted waters, which until then had drained westward into the Congo river from as far east as Lake Victoria, into a new channel which eventually reached the sea by a stream flowing northward, the present Nile.

The headwaters of the Lamia river lie high among the snow-capped peaks of Ruwenzori and when it reaches the Bwamba country below it is already a rushing stream 20 feet wide. Here it slows down on a gently sloping plateau

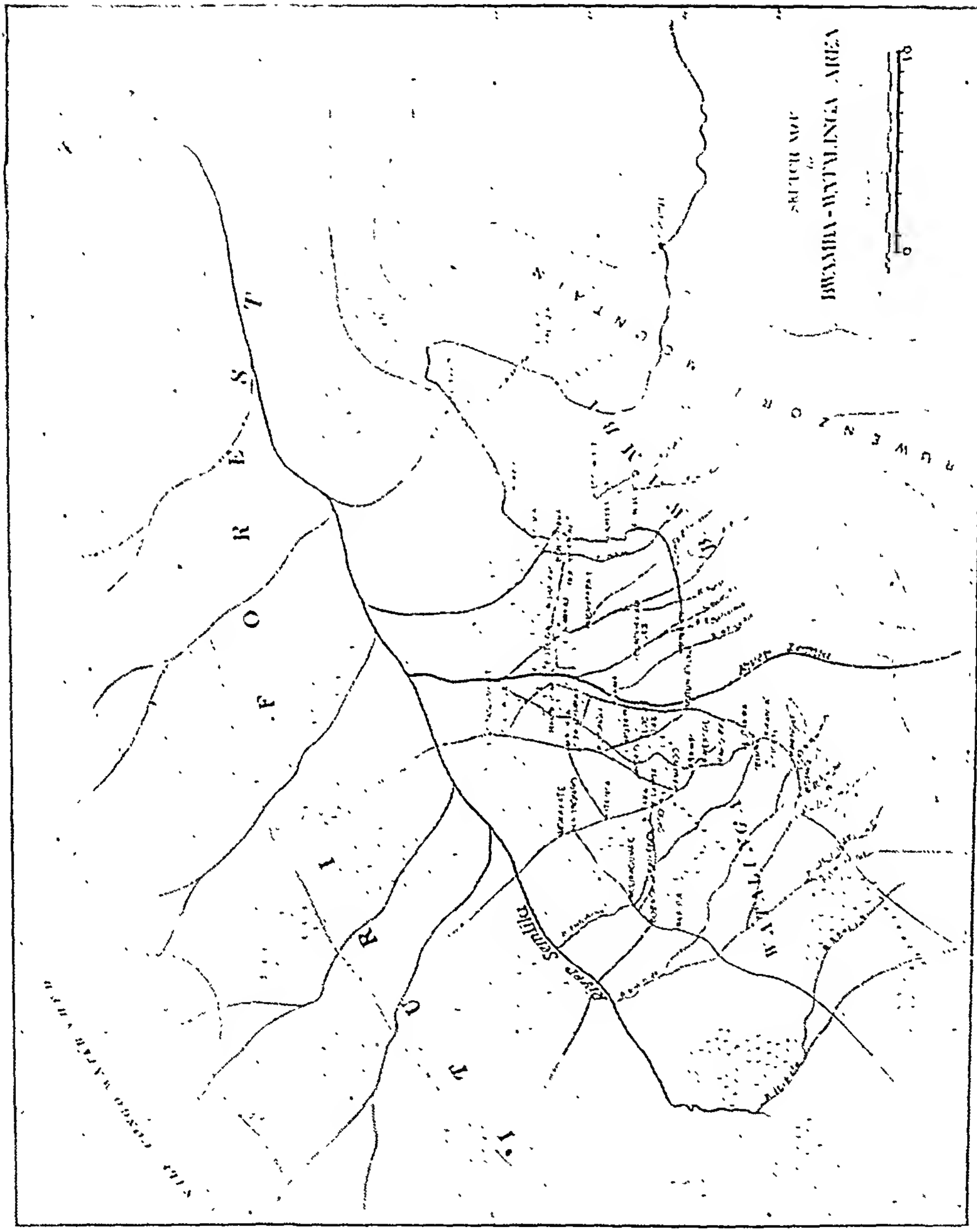
to flow for several miles in a shallow valley the sides of which are covered with grass. It then enters the forest through which it flows in a deeper trough for several more miles before joining the Semliki river. This forest is part of the great Ituri forest which begins on the northern foothills of Ruwenzori and sweeps westward across the Semliki valley, the Congo-Nile watershed, and on without interruption for more than a thousand miles to end in a ragged fringe which just fails to reach the Atlantic Ocean beyond.

The Lamia river is the largest of many small streams which carry away the water from the basin and which flow from the east into the Semliki-Albert drainage system. It has numerous small tributaries and these, like the Lamia itself, have worn deep valleys into what was once a lake bottom but is now largely forest. The terrain is consequently characterized by very deep valleys with steep sides which run parallel to one another and are separated by narrow ridges. The combination of a soft surface, a high annual rainfall (60 in.) and the nearby mountain slopes has resulted in an extreme corrugation of the Lamia basin which is highly characteristic. Rapid drainage is a feature of the area and in spite of the heavy rainfall and dense vegetation the impression is that the country is dry rather than wet.

As shown in the accompanying map the easternmost portion of the Ituri forest crosses the Lamia river just north of Butoga. The forest proper in Uganda is uninhabited except for a few nomadic pygmies. However, from the forest proper there extend outward along the streams tonguelike projections of forest which clothe the sides but not the tops of the hills. Here along the ridges, in scattered huts, situated according to personal fancy, the people reside and tend their small plantations. They depend on the streams for water and on the forest for fuel ; consequently, although they do not live in the forest, they frequently enter or pass through it. In the southern portion of Bwamba county, where the forest is replaced by grassland, and where the terrain is less rugged, the Africans still do not reside in villages. On the other hand, in the Watalinga district of the Belgian Congo just west of the Lamia river, the residents, under the influence of European administration, are all gathered together in villages, some of them quite large. In the forested portion of this district these villages are literally carved out of the forest with little more than their houses and their cultivations separating the people from the jungle. These people are, strictly speaking, inhabitants of the forest.

The flora of the Lamia basin is predominantly of the West African type although there are many plants common to both East and West Africa. The fauna is also a mixture of types. Elephant, buffalo, hippopotamus, wild pig, leopard, and rodents are numerous. Primates are represented by baboons, chimpanzees, and several varieties of monkeys including *Colobus* and *Cercopithecus*. Reptiles and amphibians are common. Among the insects, ants are most numerous but *Culicoides* and many species of mosquitoes occur in numbers.

The inhabitants of Bwamba are called the Baamba and their origin is still



BWAMBA—WATALINGA AREA.

obscure. Some authorities believe they sprang from an autochthonous race of forest pygmies; others think they represent the most primitive of the Bantu tribes in Uganda. Whatever their remote origin they show through their customs, their arts, their physical features, and their lack of tribal unity that they have long been isolated from the peoples surrounding them. They are an agricultural people and their principal cash crops are coffee and cotton. There are no horses or cattle in the area and domestic animals are confined to large numbers of sheep, goats, and dogs.

Although the time of onset of the outbreak of yellow fever in Bwamba is not known there is reason to believe that it commenced after the end of April, 1940. The virus was still active in June, 1941, as it was then that cases were seen and the virus isolated. The programme of mass vaccination instituted in August, 1941, excluded the possibility of further cases occurring after that date. The scope of the outbreak can only be roughly estimated, as the geographical limits of the affected area are not known. We do know, however, that at least six local districts, embracing a total population of about 5,000 were involved. The incidence of immunity in these localities was approximately 20 per cent. prior to the outbreak, leaving a total of some 4,000 susceptible persons. Protection tests with the sera of 168 of these individuals, taken during the epidemic, showed that 28·6 per cent. of them had become immunized. This would indicate that something over 1,100 cases of yellow fever had occurred in the six districts studied. A protection test survey carried out in August, 1941, in the Watalinga district immediately west of the Lamia river revealed that 20 per cent. of persons living in or near the forest were immune. In one village 4 miles west of Butoga five of eleven specimens gave a positive result. On the other hand 104 specimens collected from children residing in forest villages along the Congo-Nile watershed west of the Semliki valley gave completely negative results. This evidence supports the view that the outbreak was confined to a limited area in the valley of the Semliki river.

Entomological investigations in Bwamba have revealed the presence in the uninhabited forest of four species of mosquitoes known to be potential vectors of yellow fever: *Eretmopodites chrysogaster* Graham, *Aëdes* (*Stegomyia*) *simpsoni* Theobald, *Taeniorhynchus* (*Mansonioides*) *africanus* Theobald and *Aëdes* (*Stegomyia*) *africanus* Theobald. In the populated area *A. simpsoni* is by far the most prevalent mosquito. It breeds chiefly in plant axils and was found in bananas, pineapples, Dracaena and particularly in Colocasia, a widely cultivated food plant. It was not found inside houses, but in their immediate vicinity it was present in significant numbers and comprised 97 per cent. of all mosquitoes captured. *Aëdes aegypti* is rare in Bwamba and during the course of our studies only one adult was captured. A limited number of larval foci were found in tree-holes. It is obvious that *A. aegypti* has little or no importance as a vector of yellow fever in Bwamba.

These findings, together with the known fact that the incidence of yellow

fever in forested areas has been double that in the grassland portion of the country, give some indication of the mode of transmission of the disease. It seems probable that outbreaks are initiated by contact with forest vectors. Once the disease is established it is relatively certain that *A. simpsoni* plays an important role in its maintenance. The fact that outbreaks in Bwamba progress very slowly and do not cause mass immunization such as takes place during urban epidemics may be due to the habits of the principal vector as well as to the complete absence of urban centres. If this be true the possibility of more intensive outbreaks in the Congo portion of the Lamia basin would be greater, for in that district residential centres of some size do exist.

There are many gaps in our knowledge of the epidemiology of yellow fever in Africa and the whole problem requires further intensive study. The possibility of the virus being able to maintain itself indefinitely in sparsely populated or even uninhabited areas cannot be excluded. It may be that there are reservoirs of the infection as yet unknown. The lore of yellow fever epidemiology associates forest and rainfall with outbreaks of the disease in a non-urban population. The experience in Africa strongly supports the importance of both these factors but it has been found that some areas with forests and adequate rainfall manifest much less activity than others quite similar in these respects. The factors responsible for these variations in the behaviour of the virus in different areas in Africa are not at present known.

Summary.

1. An investigation covering a 4-year period has been made in an area in Uganda, East Africa, where preliminary protection tests indicated that yellow fever had occurred.

2. During the 4th year of the study an epidemic of yellow fever of some magnitude occurred in the area. Virus was isolated from a human case and from wild-caught *Aēdes simpsoni* mosquitoes.

3. The data obtained indicate that this outbreak in the human population was associated with contact with the forest. Once it became established in man the principal vector of the disease appeared to be the mosquito, *A. simpsoni*.

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THE LOCALIZATION OF THE NEUROTROPIC STRAIN OF YELLOW FEVER VIRUS IN THE CENTRAL NERVOUS SYSTEM.

BY

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In his original experiments on the formation of a neurotropic strain of yellow fever virus in mice THEILER (1930) found that after the intraperitoneal injection of adult mice the virus did not localize in the central nervous system. If, however, the mice were not more than 3 weeks old intraperitoneal injection was invariably followed by the development of a fatal encephalomyelitis. BUGHER (1941) has more recently shown that the pantropic strain of yellow fever virus as well as the neurotropic strain will penetrate to the central nervous system if injected intraperitoneally in baby mice. Very occasionally, however, an adult mouse when inoculated intraperitoneally will be found to develop symptoms of encephalomyelitis.

The method by which the virus passes from the blood into the central nervous system invariably in baby mice and very occasionally in adult mice is not without interest. Two possible routes suggest themselves. The virus may pass through the capillary walls which form the blood-brain barrier or it may be excreted on to the nasal mucosa and thence pass up through the cribriform plate to the brain. No direct evidence of the former means of spread was forthcoming although SAWYER and LLOYD (1931) found that if, in adult mice, after the intraperitoneal inoculation of the neurotropic strain the brain was traumatized

cerebral localization regularly occurred. FINDLAY and MAHAFFY (1936) brought forward some evidence to support the view that excretion on to the nasal mucosa might be followed by cerebral localization. Recently further experiments have been made which, while they do not exclude the nasal route, suggest that the yellow fever virus may pass to the central nervous system by seepage through the blood-brain barrier.

In an effort to induce poliomyelitis in rabbits SANDLER (1941) claims to have found that a hypoglycaemia induced by insulin is capable of so altering the condition of the neurones in the rabbit's central nervous system that they become sensitive to the virus of poliomyelitis injected intracerebrally. It seemed possible that by first inducing a hypoglycaemia rabbits and rats could be rendered susceptible to neurotropic yellow fever virus injected intracerebrally, a virus to which they are normally quite resistant though they produce immune bodies.

Technique.

The technique followed was similar to that used by SANDLER. Adult rabbits and rats were starved for 24 hours and 0·6 to 0·8 units of insulin per kg. of body weight were injected subcutaneously. Two hours later four rabbits were inoculated intracerebrally with 0·2 c.c. of a 20 per cent. suspension of infected mouse brain in serum saline (1 in 10): six rats received 0·08 c.c. intracerebrally. No rise in temperature and no nervous symptoms appeared in any of the animals.

Encephalomyelitis in Mice

As a control to the above animals a batch of six fully grown mice, starved for 24 hours were given 0·8 units of insulin per kg. of body weight subcutaneously and 2 hours later 0·2 c.c. of a 20 per cent. suspension of infected mouse brain intraperitoneally. Five of these six mice subsequently developed symptoms of encephalomyelitis.

In view of this result further investigations were carried out on mice.

NUMBER OF DAYS FROM INTRAPERITONEAL INOCULATION TO DEVELOPMENT OF SYMPTOMS.

Number of Experiment.	Insulin.	Normal Control.
1	5, 5, 6, 8, 10, –	– – – – – – – –
2	5, 6, 6, 10, – –	– – – – – – – –
3	5, 5, 5, 5, 6, –	6 – – – – – – –
4	5, 6, 6, 6, 7, 9	– – – – – – – –
5	6, 6, 8, 12, 13, –	– – – – – – – –
6	5, 5, 5, –, –, –	– – – – – – – –
7	6, 8, 8, 10, –, –	– – – – – – – –

The results in the table show that thirty-two out of forty-two mice developed encephalomyelitis after intraperitoneal inoculation of neurotropic yellow fever virus when they had been starved and subsequently injected with 0·6 to 0·8 units of insulin per kg. of body weight. In experiments 3 and 4 the insulin was given in two doses 0·4 units per kg. of body weight being injected 2 hours before the intraperitoneal inoculation of the virus, 0·4 units 3 hours later.

That the results were due to the prolonged hypoglycaemia and not to any direct toxicity of the insulin was shown by the fact that if the mice had been fed 2 hours before the insulin injection there was no prolonged prostration and subsequently no localization of virus in the central nervous system. Doses of insulin such as 0·2 units per kg. of body weight which did not produce so prolonged or so profound a hypoglycaemia did not have any effect on localizing virus in the central nervous system.

A clue to the mechanism by which insulin shock may act in localizing virus in the central nervous system is given by the work of DAMESHEK, MYERSON and STEPHENSON (1935) who noted that in insulin shock there is a marked diminution in the arterio-venous difference in oxygen content : this diminution may signify actual diminution in oxygen uptake by the tissues. The neurological symptoms associated with hypoglycaemia due to insulin may thus be due to the effects of lack of oxygen in brain tissue. Arguing on these lines it appeared to be of interest to determine whether lack of oxygen in the blood would localize the neurotropic strain of yellow fever virus after intraperitoneal inoculation. Ten adult mice were therefore exposed to coal gas so that they became unconscious for approximately 10 minutes : 30 minutes later the virus, 0·2 c.c. of a 20 per cent. suspension of infected mouse brain, was injected intraperitoneally and the gasing was repeated. Four of the treated mice developed yellow fever encephalomyelitis in from 5 to 8 days : the ten control untreated mice similarly injected with neurotropic virus remained in good health. Treatment with coal gas was thus capable of localizing virus in the central nervous system, in addition to insulin shock. In order to make certain that the virus localized in the brain was that of yellow fever and not a virus carried by the mouse, neutralization tests were performed with a known yellow fever immune serum and the infected brains. Histological examination of brains was also carried out. The results showed that the yellow fever virus was responsible for the development of the encephalomyelitis.

DISCUSSION.

The experiments here described show that insulin hypoglycaemia sufficient to produce nervous symptoms and coal gas poisoning are able to localize a virus in the central nervous system. It is suggested that the localization is due to seepage through a capillary wall which is damaged possibly by lack of oxygen.

In this connection it is of interest to note that on rare occasions in man nervous sequelae have followed an acute attack of yellow fever. In ordinary

yellow fever owing to the liver necrosis there is a profound and prolonged hypoglycaemia.

CONCLUSIONS.

Experiments are described to show that after intraperitoneal injection of the virus insulin shock is capable of localizing the neurotropic strain of yellow fever virus in the central nervous system of adult mice. Coal gas poisoning may produce a similar result. It is suggested that owing to deficiency of oxygen the blood-brain barrier is damaged and seepage of the virus occurs.

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A PRELIMINARY REPORT OF AN OUTBREAK OF KALA-AZAR IN A BATTALION OF KING'S AFRICAN RIFLES.

BY

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Introduction.

The occurrence of an outbreak of kala-azar in a K.A.R. battalion which has been operating in Northern Kenya is described. This preliminary report is presented, in its present incomplete form, because although kala-azar has been adequately described in the Sudan, little is known of the disease in Kenya or in Abyssinia. Now that bodies of troops are moving about these areas, it is hoped that a description of this outbreak may make clear the possibility and the danger of infection and may help in diagnosis at a more early stage of the disease.

Thirty cases of the disease have occurred in the battalion in question and one in an auxiliary unit; and since all were under medical care from the outset a more accurate picture of the disease can be obtained than would be possible from the study of sporadic cases entering civil hospitals. The entire

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The clinical observations were made by the first two authors (A.C.E.C. and P.C.C.) and the laboratory observations by the third (G.R.).

history of the unit can be traced and the time of entry into the probable area of infection is known as well as the approximate date of the onset of symptoms. In these circumstances the length of the incubation period can be assessed with a fair degree of accuracy.

The study of these cases has revealed certain inconsistencies in the usual description of kala-azar and definite difficulties in laboratory diagnosis in the early stages of the disease. So far in only some of the cases of the series has the diagnosis been confirmed by the discovery of the parasite, while only a few have been cured.

Though literature on the subject is not available it is felt that there is sufficient justification for presenting this preliminary report.

History and Epidemiology.

The battalion was recruited in July, 1940, mainly from the Wakamba and Kipsigis tribes. It was in training in the Nairobi area until 5th December, 1940. No cases of kala-azar have been reported from these areas during the past five years. From 5th to 12th December the battalion was travelling to the northern Lake Rudolph area (Turkana) and reached Kalin on the 12th. It remained in the area Kalin-Lokitaung until 10th February, 1941. This district is dry and there were no mosquitoes, but flies were numerous.

On 10th February it moved to the area Todenyang-Namaraputh on the shore of the northern end of the lake, near to a point where the frontiers of Kenya, the Sudan and Abyssinia meet. Here there are grassy mudflats and reeds, the heat is humid and flies and mosquitoes occur.

From 6th to 19th March the battalion moved in convoy to Nanyuki and then returned to Kalin, where it remained one week.

On 30th March it moved to Kibesh Wells on the Kibesh river, a humid district with numerous mosquitoes, both anopheline and culicine.

" A " Coy. of the battalion remained at Kibesh Wells not more than three nights and then moved on to Washwaha at the foot of the Maji escarpment.

The battalion moved to Todenyang on 18th May, except " D " Coy., which went to Kalam, a district 20 miles north of Todenyang in low lying ground near the Omo river, and " B " Coy., which was at Shunguru.

On 1st June the whole battalion concentrated at Todenyang and then returned by Lokitaung and Kitale to Nyeri.

Admission and Diagnosis.

When the battalion became exposed to the cold and damp of its camp near Nyeri there was an outbreak of respiratory disease of influenzal type, and a number of cases of malaria also declared themselves. In addition to these, from the second half of June onwards, there were admitted to hospital a number of cases which did not conform to any known type of disease.

These were characterized by certain clinical and pathological features as follows :—

1. High pyrexia (104–105° F.) continuous or remittent.
2. Lack of toxaemia or malaise such as might be expected with hyperpyrexia.
3. Negative blood cultures, absence of blood parasites and negative serum reactions for enteric, brucella or typhus fevers.
4. Enlargement of the spleen of varying degree from the barely palpable to the gross.

To the time of writing, thirty-one cases have been admitted. The date of onset of symptoms has ranged from 11.6.41 to 30.8.41. Further cases may yet occur.

In twenty-two of these cases leishmania were discovered. There have been fourteen deaths so far (November, 1941). In all of these fatal cases leishmania had been demonstrated. Some cases (three proved and six unproved) have been discharged cured or on sick leave. These figures show the seriousness of the disease, both in mortality and length of hospitalization.

CASES ADMITTED TO HOSPITAL.

Company.	Number of Cases.	Number in which Leishmania were found.	Deaths.
H.Q.	7	5	3
A.	1	1	1
B.	6	4	3
C.	3	1	Nil
D.	13	10	7
2(K)MAC	1	1	Nil
Totals ...	31	22	14

DISTRICT IN WHICH INFECTION WAS ACQUIRED

The heaviest incidence has fallen on " D " Coy., with " H.Q." and " B " Coy. next in order. If "H.Q." Coy. be disregarded because it was generally operating in a scattered manner so that its component parts may well have moved over a wide area, it may, perhaps, be significant that " D " and " B " Coys. were on detached duty, the former in Kalam and the latter in Shunguru. Both these districts are in low lying country near the Omo River.

" A " company's comparative immunity (one case) may not be unconnected with the fact that it remained at Kibesh Wells for not more than three nights and then moved to Washawaha at the foot of the Maji escarpment.

The following investigations have been carried out by Colonel MACLEAN and others.

1. A survey of the native population revealed microscopically positive cases at Shunguru and Kibesh Wells, and a number of other persons with illness and enlarged spleen and liver.

2. A sandfly (*Phlebotomus*) survey of the area from Lodwar to Kibesh Wells showed a dry season sandfly infestation of the whole of the Sanderson Gulf area (north of Lake Rudolph) in the vicinity of rivers, particularly at Kalam, Shunguru and Kibesh Wells. The infestation dropped rapidly as the distance from the river increased.

It is noteworthy that no European cases have occurred. A possible explanation is that in drier areas sandfly infestation only occurred or was more intense in the shade of native huts and in the organic pollution around them. African troops would be more likely to frequent these places.

Incubation Period.

Probably the first opportunity for infection was in the Todenyang-Namarabuth area from 10th February onwards.

The date of onset of symptoms in the first recorded case was 11th June, 1941. The symptoms of a number of the subsequent cases started between 26th and 28th June. This would seem to indicate that the incubation period is somewhere in the neighbourhood of 4 months. Confirmation of this figure may be obtained in another way. The first seven cases occurred between 11th and 29th June and were distributed between all five companies, and this probably corresponds with an infection occurring in the period 10th February to 6th March, when the whole battalion was in the Todenyang-Namaraputh area.

Later cases from 7th July to 28th August were distributed as follows: "D" Coy., 12; "B" Coy., 3; "H.Q." 3. It is not unreasonable to suppose that this corresponds with infection during the period 18th May to 1st June, when "D" Coy. was on detached duty at Kalam. In this case the incubation period would appear to be about 2 months.

CLINICAL FEATURES AND PATHOLOGY.

Onset.—The onset was sudden with headache and high fever rising above 103° F. in 24 hours, except in four cases where there was a gradual rise of temperature during the first week, not reaching the maximum until the second week.

Fourteen cases complained mainly of abdominal pain, five of them vomiting. The pain was sometimes over the spleen or liver but more often had a general upper abdominal distribution. Only one case complained of diarrhoea but four others were found to have loose stools containing pus and blood cells.

Seven cases complained of cough and pain in the chest, or both, and these were found to have some physical signs such as an occasional rhonchus or a few râles at one base. Three cases complained mainly of pain in the neck, two had definite stiffness amounting to rigidity. Four cases complained mainly of sore throat. On examination of the throat there was little to see beyond the general appearance of a moderate degree of pharyngitis.

The Temperature.

The course of the fever in these cases is variable, one constant feature being the high temperatures registered: 104–105° F. at one stage or another of the disease.

Some cases commenced with a high almost continuous fever in the first week, changing to a more remittent type in the second, and perhaps dropping in level in the third to a comparatively moderate 100–102° F. In others the temperature gradually climbed in the first week as in typhoid and remained high in the second. Even without treatment there seems to be, in some cases, an undulant tendency—the temperature dropping to 99–100° F. and perhaps rising again after another week or two. This fall in temperature may be sometimes wrongly attributed to therapeutic measures.

Two or more peaks of fever in the 24 hours were not infrequently observed.

Splenomegaly and Hepatomegaly.

In all descriptions of kala-azar extreme enlargement of the spleen is one of the points emphasised. On admission, however, no less than seven cases had no detectable splenic enlargement while in a number of others the spleen was only just palpable. The number of cases which on admission had slight hepatic enlargement was seven, but on subsequent examination 4–6 weeks later this number had increased to twelve. Six weeks after admission there were only two patients in whom the spleen was not palpable and in the remainder the average size of the organ, as measured in fingers' breadth below the costal margin, had increased. It would appear that while the spleen may enlarge before febrile symptoms occur, it requires some weeks or months of illness for this to become outstanding. It is important to note that the failure to detect enlargement does not exclude kala-azar.

Kidneys.

A point that seems to emerge from a study of these cases is that damage to the kidneys usually occurs. Out of twenty-seven cases where the urine was tested, twenty-four showed albumin present as a fair cloud, and in fifteen of these granular casts were also present. At postmortem examination of the fatal cases the kidneys were found to be swollen under the capsule.

Clinical Appearance.

The majority of the cases have been characterized by a remarkable appearance of fitness in spite of the fever. Thus, a man with a temperature of 103° F. might be seen walking around the ward and making a pretty fair effort at eating his food ration. On interrogation, he would reply, as often as not, that he had no complaint of any sort beyond a certain diminution of his customary vigour, or he might complain, in addition, of a slight headache.

Examination would show a clean tongue and few physical signs beyond fever, some degree of pallor of the mucous membranes and, in some cases, splenomegaly. In sharp contradistinction to the above, four of the cases were admitted in a definitely typhoidal state, with dirty tongue and apathetic manner. Later, however, the condition of those showing few symptoms might deteriorate seriously.

Blood Counts.

White Cell Count.—The white cell count is included here as it seems to be one of the chief characteristics of the disease and is especially valuable for diagnosis. It is invariably lowered even in the very earliest stages of the disease and a normal or high count excludes a diagnosis of kala-azar. The total white counts of the cases studied in hospital varied between 1,400 and 6,200, with an average of 3,340 w.b.c. per cu. mm.

Even more striking is the change in the polymorphonuclear cells, for not only is there a drop in the total number but the normal ratio of 2/3 polymorphs to 1/3 lymphocytes is completely reversed.

The differential polymorphonuclear count averaged 36 per cent., with variation between 18 and 56 per cent. In two cases complete agranulocytosis occurred at one point. This granulocytopenia is undoubtedly the cause of some of the complications of the disease.

Haemoglobin and Red Cell Count.—There is nothing characteristic in the red cell count. Anaemia is frequent and may be extreme but is not invariable. It appears to be hypochromic in type. On admission, haemoglobin varied between 30 and 90 per cent. and unless treated it tended to drop further as the disease progressed, eventually reaching very low levels.

Complications.

The more important complications noted were as follows :—

1. *Haemorrhage.*—There appears to be an increased tendency to bleed from mucous surfaces, although skin petechiae were only once seen. It appeared very commonly as a terminal manifestation. Epistaxis occurred in five cases, once terminally. Haemorrhage into or from the gums occurred in four cases. Haematemesis and urethral haemorrhage occurred once each. In five cases severe bowel haemorrhages occurred terminally.

2. *Diarrhoea.*—Although only one case complained of dysenteric symptoms on entry, four others were found to have blood and pus in their stools. Diarrhoea occurred at some stage or another during every patient's illness, and recurred frequently in those cases not doing well.

Five fatal cases had severe bloody dysentery amounting to bowel haemorrhage as a terminal symptom.

The administration of therapeutic tartar emetic frequently has the effect of producing diarrhoea.

3. *Pharyngitis.*—Sore throat, dysphagia, and cough with the expectoration of muco-pus occurred in fifteen cases at one time or other during their illness. The throat appearances were indefinite—the pharynx appearing dryish, granular or gelatinous. This condition is probably associated with granulocytopenia.

4. *Pneumonia and Bronchitis.*—In addition to the seven cases complaining of cough and pain in the chest, four others were found during their illness to have rhonchi or râles in their chests—presumably due to bronchitis.

Two cases developed a definite pneumonia and recovered. In one a white cell count of 2,000 (all lymphocytes) improved to 3,600 with 30 per cent. polymorphonuclears ; in the other no alteration occurred.

Other Features Noted.

Glandular Enlargement occurred in seven cases, generally only the femoral or inguinal group. A generalized enlargement was found in two cases, in both of which the Kahn test was negative.

Skin Rashes.—A fine papular eruption of the face was noted in four cases. In one, scraping of a papule showed leishmania associated with pus cells, while culture yielded a growth of cocci.

Terminal Condition.

Inanition (five cases).—Four patients died in a state of inanition resembling typhoid, in which there was a gradual loss of vitality till death occurred ; a fifth died with a combination of terminal pneumonia and inanition.

Heart Failure (three cases).—One died unexpectedly of collapse and heart failure while comparatively well ; another of heart failure following a serous pericarditis ; a third had a pericarditis of pneumococcal origin associated with pneumococcal septicaemia. In all fatal cases the heart had a flabby, oedematous or gelatinous appearance.

Dysentery (four cases).—Four had severe dysentery with considerable bowel haemorrhage as a terminal phenomenon ; one had in addition bronchopneumonia, and one epistaxis.

Epistaxis (two cases).—Two cases presented epistaxis as a terminal phenomenon, one in addition suffering from haemorrhagic dysentery.

Delirium.—One died in febrile delirium.

Results of Treatment.

These have been very disappointing. The only antimonial drug available was sodium antimonyl tartrate. Tryparsamide was also tried but with no success.

Tartar emetic was used in a course of about 25 to 27 grains in 12 to 14 intravenous injections on alternate days. In every case it succeeded in bringing the temperature down to normal or nearly so, but on stopping it the fever usually returned after 2 or 3 weeks except in the few successful cases.

A second course might again reduce the fever, but the condition of the patient might then have deteriorated to such an extent that he might not be able to tolerate the relatively toxic drug.

Some got tired of injections and flatly refused further treatment.

So far three cases diagnosed microscopically have been discharged to sick leave, and six unconfirmed cases have been discharged.

Clinical cure in these cases merely consisted in absence of fever for 3 or more weeks, and in general well-being, gain in weight etc. The spleen did not decrease markedly in size, and in the confirmed cases a second examination for leishmania was not done. The blood count remains abnormal with low haemoglobin red cell count, and also a low white cell count with relative lymphocytosis.

Laboratory Investigation.

Diagnostic Puncture.—The spleen, liver, bone marrow, and tonsils were all, in one or other of the cases, submitted to diagnostic puncture. Superficial scrapings of the skin were also examined but with negative result. The parasite was found in the spleen, liver, bone marrow and lymph gland, the spleen having yielded the most successful results.

Parasites are very scanty in the early stages and it is only later in the disease that they can be demonstrated more easily. In every postmortem examination careful search showed a few parasites in the spleen or liver or bone marrow, although puncture during life often did not collect enough tissue to reveal them.

Material from thirty-one cases was examined in the Laboratory, comprising :—

		Cases.			Cases.
Spleen smears	... in	11	Blood culture	... in	10
Liver smears	... ,,	6	Spleen culture	... ,,	2
Bone marrow smears	,,	8	Postmortem material	,,	11
Lymph gland smears	,,	3	Tonsil and nasal swabs	,,	6

Leishmania were found in twenty-two cases.

Spleen Smears.—Seven showed parasites and in only one case were they at all numerous. Usually they were extremely scanty and found only after a prolonged search (1 hour in some cases).

Liver Smears.—Four were positive. The parasites were scanty.

Bone Marrow Smears.—One only showed very scanty extracellular parasites.

Lymph Gland Smears.—Two out of three examined showed very scanty parasites.

Blood Cultures.—None was positive. The media used for blood culture were: (1) N.N.N., using human blood. (2) Citrated blood. (3) A mixture of laked blood and 1·2 per cent. saline.

Spleen Culture.—Material from two postmortems was cultured in citrated blood medium. In one case flagellate forms were seen on the 17th day. They gradually increased in number for about 14 days when the culture became contaminated. The body from which the culture was made had been lying in the mortuary for 18 hours. The second culture was contaminated from the start.

Postmortem Material.—Parasites were found in smears of spleen and bone marrow in all cases which came to postmortem. They were very scanty and on this account were not seen in sections stained with haematoxylin and eosin. They were found in liver smears in three of the cases. In one case of kala-azar (not in the present series) parasites were very numerous in sections of spleen liver and lymph glands.

Tonsillar and Nasal Swabs.—Smears from six cases were examined. All were negative.

Skin Scrapings.—Smears from a proved case (spleen puncture) were examined. No leishmania were seen. In another case leishmania were found in scrapings from a facial papule.

Appearance of the Parasite.

In most cases the parasites were scanty and often distorted. They were rarely intracellular.

Four types were seen :—

(*a*) A round swollen parasite with well-marked body and nuclei.

(*b*) An elongated parasite in which the body and nuclei stained well.

(*c*) A parasite in which the body did not show up at all. All that could be seen being the trophonucleus and kinetoplast. A group of these in a cell gave a very characteristic picture.

(*d*) The typical text-book parasite. These were scarcely ever seen.

The above appearances were seen in both Leishman and Giemsa stained preparations.

Generally speaking the kinetoplast was very conspicuous and drew one's attention to the parasite.

It must be emphasised that prolonged search is necessary before reporting a smear as negative.

Some of the distortion and bad staining may have been due to a little of the local anaesthetic getting into the needle. For this reason the later punctures were done without an anaesthetic and the results were noticeably better.

Formol-gel Reaction.

This test was done in twenty-one cases in the present series and was completely negative. The test was positive in one case from another unit in whom the disease was of long standing (7 months).

Miscellaneous Postmortem Appearances.

In all cases the heart was pale and flabby and the myocardium oedematous.

The kidneys in two cases showed necrosis and partial disintegration of the epithelium of the convoluted tubules. All cases showed oedema of the perinephric tissue, and cloudy swelling of the kidney.

Splenomegaly was a feature of all the cases, though frequently not very marked.

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ENDEMIC TYPHUS IN SOUTHERN NIGERIA.

BY

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Nearly every year since 1920 there have been references of one kind or another to the occurrence of cases of typhus or "typhus-like" diseases in Nigeria. In the Annual Medical Reports for the Colony the references have been to "typhus" or "typhus-like diseases" or to a "positive Weil-Felix reaction." It has not been found possible to follow up these statements to correlate the clinical and pathological data supplied.

There are, however, two series of published cases which demand attention. Both were published in a journal which, unfortunately, is now defunct. Under the heading of "Notes upon the occurrence of a 12-day fever of the dengue group in Nigeria," Davies and Johnson (1921) published an account of eighteen

* We wish to express our gratitude to Dr. E. C. Smith, Medical Research Institute, Yaba, for much help and to the Acting Director of Medical Services, Nigeria, for permission to publish this paper.

cases of continuous fever characterised by the following features. The fever lasted 10 to 13 days and fell by lysis; there was a maculo-papular rash; in some cases suffusion of the eyes was noted; the pulse was relatively slow. In six cases the "Widal reaction was positive," but all had been inoculated against typhoid. There is no record of a Weil-Felix reaction. Fifteen of the cases were Europeans, three were Africans. The evolution of the fever and rash and the absence of any "saddle-back" feature in the temperature charts illustrated is suggestive of typhus rather than dengue.

NAUDI (1938) and DAVEY (1938) described two similar cases in Europeans from Kano. In both there was a 14-day fever with muscular pains, relatively slow pulse and a maculo-papular rash. The eyes were suffused. In one of the cases the Weil-Felix reaction performed at Lagos on blood taken on the 12th and 14th days of the fever showed agglutination within normal limits. In the other a Weil-Felix reaction performed by DAVEY 1 month after recovery gave a trace of agglutination for *Proteus* X19 at 1/1280, and 4 months later the titre had fallen to a trace at 1/640. This case was complicated by agglutination for paratyphoid A at 1/800, but as the patient had been previously inoculated with T.A.B. vaccine this is possibly an "anamnestic" rise in titre, as DAVEY points out. In both series of cases it should be noted that the patients came from Northern Nigeria—a part of "Hausaland" which lies between the Sahara in the North and East and the dense jungle belt along the Bight of Benin in the South and West. The following two cases have been met with in Lagos (Southern Nigeria) within the past few months.

CASE 1.

D.A.A., African male, aet. 32, a labourer dealing with cattle. Admitted 9th November, 1941; discharged, 28th November, 1941.

Complained of generalised pain, more especially backache, joint pains with fever of 4 days' duration. He had been taking quinine for the fever.

On examination the positive findings were: a drowsy, rather apathetic facies, clean tongue, enlarged spleen—two fingers—and tenderness of the leg muscles.

Laboratory Findings.

Urine.	A trace of albumin and a few pus cells.
Stool.	A few ascaris ova.
9.11.41.	Thick blood film—negative.
11.11.41.	" " " —a few S.T. rings.
12.11.41.	W.B.C. 14,600.
13.11.41.	W.B.C., 13,900; polys., 61 per cent.; lymphs., 29 per cent.; monos., 7 per cent.; eosins, 3 per cent.; blood culture, negative.
19.11.41.	Blood culture—negative.
26.11.41.	C.S.F. cells, 63 all monos. (some r.b.c. present); protein, 60 mg. per cent.; globulin, no excess; chlorides, 750 mg. per cent.

Guineapig inoculated from C.S.F. healthy after 14 days.

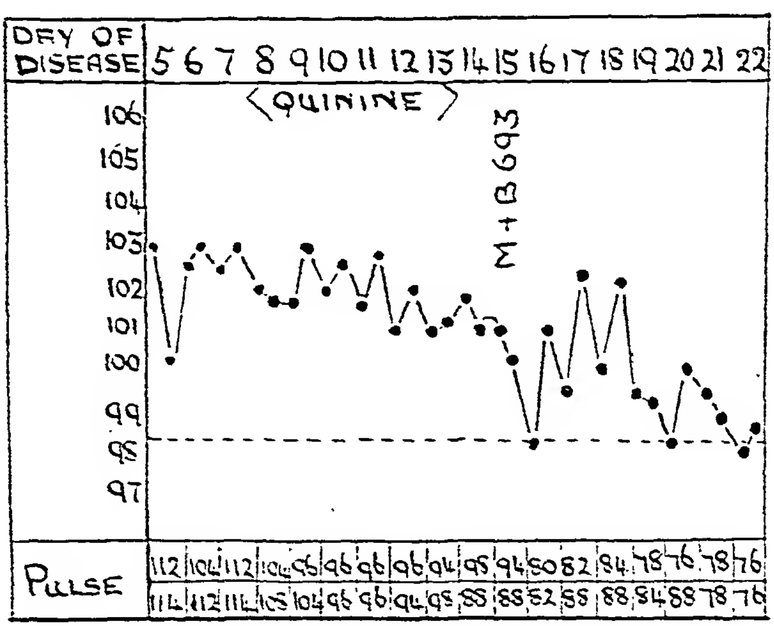
Treatment included quinine—10 grains i.m.—on 12.11.41, followed by 10 grains t.d.s. orally for 5 days. It had no obvious effect on the fever. By an oversight six tablets of M. & B. 693 were prescribed and taken on 19.11.41. The results of various agglutination tests are given in the following table:—

TABLE I.
RESULTS OF AGGLUTINATION TESTS (CASE I).

Date.	Typhoid	Paratyphoid A	Paratyphoid B	Paratyphoid C	*Brucella abortus.*	*Brucella melitensis.*	OXK	*Proteus* OX2	OX19	Kahn Test.
11.11	nil	nil	nil	nil			Not performed			
14.11	nil	nil	nil	1/25	nil	nil	Not performed			
17.11			Not performed				1/40	1/80	1/960 (trace)	++++
27.11			Not performed				1/40	1/160	1/1280 (trace)	
13.12	nil	nil	nil	nil	nil	nil	1/40	1/40	1/320 (trace)	
Reading after 2 hours incubation at 56° F.							Reading after incubation overnight.			

All suspensions used were obtained from the Standards Laboratory, Medical Research Council, Oxford.

Progress. Fall in temperature after M. & B. 693. There was no other change until the fever declined on 25.11.41. Within two days there was a dramatic change in outlook, the previously apathetic patient becoming bright and cheerful and clamouring for discharge. The temperature chart is reproduced below.



The patient left Lagos after discharge.

Case 2.

African male, aet. 18 years, domestic servant. Admitted 11.12.41. Had been in Lagos 1 month before illness began.

Complained of pain chiefly in chest with some cough and fever of 1 week duration.

On examination, tongue was slightly coated, spleen enlarged. Apathy and drowsiness were noted from the beginning. Backache was severe occasionally. The course of the disease was very similar to that in Case 1. The rapid convalescence was again especially noted.

The laboratory data of interest are given below:—

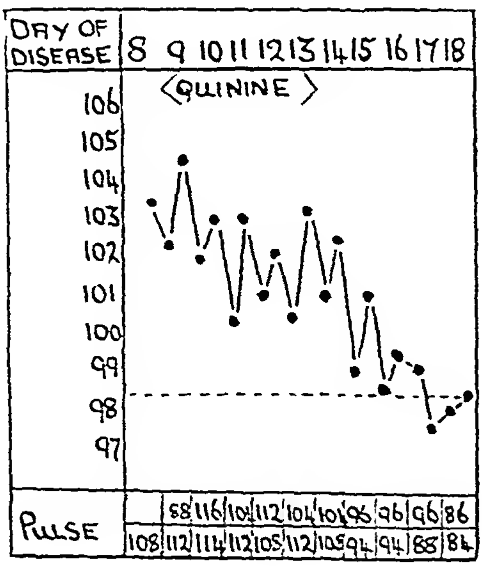
11.12.41. Blood—no malaria parasites.
13.12.41. W.B.C., 4,200; polys., 58 per cent.; lymphs., 36 per cent.; blood culture, negative.
13.12.41. C.S.F. Cells, 2; protein, 40 mg. per cent.; globulin, no excess; chlorides, 720 mg. per cent.

The agglutination results (Table II) and temperature chart are reproduced below:—

TABLE II

RESULTS OF AGGLUTINATION TESTS (CASE 2).

Date.	Typhoid H	Typhoid O	Paratyphoid A	Paratyphoid B	Paratyphoid C	OX19	*Proteus* OXK	OX2	Kahn Test.
13.12	nil	1/20	nil	nil	nil	1/80	1/40	1/20	Not done
18.12	nil	nil	nil	nil	nil	1/640	1/40	1/40	++++
24.12	Not performed					1/1280	1/80	1/160	++++



COMMENTS.

The cases described leave little doubt that in Nigeria there exists endemic typhus. Very characteristic of typhus from the clinical side are the lethargic, rather drunken facies, and the rapid convalescence as soon as the temperature drops. There is a close clinical similarity between these cases and some cases of "urban" typhus seen by one of us (W.H.) in Malaya.

In both instances here reported the subjects had been living in the township of Lagos. This, taken with the absence of primary eschar and the high X19 agglutination, suggests a flea or louse-borne rather than a tick or mite-borne typhus. The cases previously reported came from Northern Nigeria where the infestation with lice is much higher than in the South—high enough, indeed, to sustain periodic epidemics of relapsing fever. Nevertheless, the high incidence among Europeans in both series and the endemic rather than the epidemic nature of the disease are points against the louse as vector. The absence of a rash in our two cases might seem to differentiate them from the two series previously published. It is to be noted, however, that the previous authors dealt either with Europeans or comparatively light-skinned natives of the North in whom a rash is easy to detect. The lethargic facies which we noted in our cases is partly to be attributed to the encephalitis of typhus but it might also conceal a rubeolar rash in the deeply pigmented skin of the Southern negro. In so far as it is safe to generalise from such a few cases,* we believe that the disease in our series from the South, and those of DAVIES and JOHNSON and NAUDI from the North of Nigeria is identical—endemic typhus, probably flea-borne.

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* Since writing the above we have met with two further cases of typhus from Lagos giving an agglutination of *Proteus* X19 up to 1/640.

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MALARIA AND THE MUD LOBSTER

BY

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AND

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The burrowing activities of the mud lobster, *Thalassina anomala* (Herbst), Fig. 1, have long been recognised as a hindrance to engineers engaged in constructional work in swampy areas near the sea. Its relation to malaria control in such areas is less widely recognised, though this may be quite as serious. The burrowing may render ordinary anti-malarial measures ineffective, because of the unsuspected breeding places which the mud-holes provide, as well as through the damage of undermining caused to tidal bunds.

Experiments have been undertaken in Singapore with the object of evolving a cheap and reliable method of destroying the animals and also of preventing their burrowing in tidal bunds. The investigations have been centred on a tidal zone with a sea-frontage of about 2 miles at Changi, a district on the east coast of Singapore Island. Neighbouring areas equally infested by the mud lobster, but where the danger of mosquitoes and malaria could be ignored, were available for comparison while the experiments were being carried out.

* This note, which was completed shortly before the Japanese invasion of Malaya, was brought to London by the senior author. Mr. TWEEDIE is unhappily posted as missing and is believed to be a prisoner of war.

Excreta were obtained from seventeen of the positive Vi reactors, and from six of these subsequent samples of sera were also obtained for repeated agglutination tests. Three whose sera were tested on three occasions at monthly intervals all yielded the same titres in the second test as in the first, but in the third test no agglutination occurred. Of three whose sera were examined on only 2 successive months, one continued to show the persistence of agglutinins, while two were negative on the second occasion.

TABLE.

VI AGGLUTININS IN 1,042 RANDOM SERA.

Agglutination titres against Strain "Vi 1"	Number of Sera.	Percentage showing Agglutination.
1 in 5	52	4·99
1 in 7·5	4	0·38
1 in 12·5	1	0·09
1 in 25	2	0·19
Total 1 in 5 - 1 in 25	59	5·66

Excreta were cultivated from seventeen of the positive Vi reactors, two were examined on three occasions at monthly intervals, two on two occasions and the remainder on one occasion only. From only one case was *Bact. typhosum* isolated, the organisms being found in the stools on both the two occasions they were examined. The sera from this were also examined on two occasions and each time the Vi agglutination titre was 1 in 5.

These findings serve to support the contention that the presence of low titre typhoid Vi agglutinins in an African population are widespread and not necessarily indicative of the existence of the carrier state.

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ANNUAL GENERAL MEETING

of the Society held at

Manson House, 26, Portland Place, London, W.,

on

Thursday, 23rd July, 1942, at 4.30 p.m.

THE PRESIDENT

Sir RICKARD CHRISTOPHERS, *C.I.E.*, F.R.S., Colonel I.M.S. (ret.), in the Chair.

BUSINESS.

REPORT OF THE COUNCIL FOR THE YEAR ENDED 31ST MARCH, 1942.

The **Hon. Secretary**, Dr. C. M. WENYON, presented the Thirty-fifth Annual Report, copies of which were circulated at the meeting.

The adoption of the Report was duly proposed, seconded and carried.

REPORT OF THE HON. TREASURER FOR THE YEAR ENDED 31ST MARCH, 1942.

The **Hon. Treasurer**, Dr. O. MARRIOTT, presented his report, together with the Accounts and Balance Sheet prepared by the Auditors, Messrs. W. B. Keen & Co., and approved by the Audit Committee.

Dr. MARRIOTT said he considered the financial position satisfactory in face of present difficulties.

He referred to the inevitable loss of rents and of certain Fellows' subscriptions, though he was glad to note that forty-five new Fellows had joined the Society, including one Life Fellow.

On 31st March, 1942, the debt on Manson House stood at £8,581 19s. 2d., having been reduced by £316 2s. 9d. during the year with the help of various donations for that purpose.

The Treasurer's Report was adopted by the meeting.

ELECTION OF AUDIT COMMITTEE.

The Audit Committee, consisting of Dr. V. S. HODSON, Col. F. P. MACKIE and Dr. W. E. COOKE, was re-elected.

Election of President, Vice-Presidents and Twenty Councillors.

In view of war conditions, it had been considered advisable to postpone again the usual postal ballot for election of a new President, Vice-Presidents and Council. The Council had therefore nominated for re-election at the Annual General Meeting those at present holding office, together with Air Marshal Sir Harold Whittingham as Vice-President in place of the late Dr. A. J. R. O'Brien; and Dr. A. G. H. Smart, of the Colonial Office, and Dr. Charles Wilcocks, of the Bureau of Hygiene and Tropical Diseases, to fill the two vacancies on the Council.

The proposals were then put to the meeting and the Fellows present unanimously agreed to the election of those nominated, namely:—

President:

Sir S. Rickard Christophers, *C.I.E.*, *O.B.E.*, F.R.S., Col. I.M.S. (ret.).

Vice-Presidents:

Sir Sheldon F. Dudley, *K.C.B.*, K.H.P., M.D., F.R.C.P., D.T.M. & H., F.R.S., Surg.-Vice-Admiral R.N.

Robert Priest, *C.B.*, K.H.P., M.D., F.R.C.P., D.T.M. & H., Major-General A.M.S.

Sir Harold Whittingham, *K.B.E.*, K.H.P., D.T.M. & H., F.R.C.P., Air Marshal R.A.F.

Councillors:

D. B. Blacklock, *C.M.G.*, M.D., D.P.H., D.T.M., Professor.
C. C. Chesterman, *O.B.E.*, M.D., B.S., M.R.C.P., D.T.M. & H.
J. A. Cruickshank, *M.C.*, M.D., D.P.H., Major I.M.S. (ret.).
N. Hamilton Fairley, *O.B.E.*, M.D., D.Sc., F.R.C.P., D.T.M. & H., F.R.S., Colonel A.A.M.C.
G. W. M. Findlay, *C.B.E.*, M.D., D.Sc., Brigadier A.M.S.
Clifford A. Gill, M.R.C.P., D.P.H., D.T.M. & H., Col. I.M.S. (ret.).
R. M. Gordon, *O.B.E.*, M.D., M.R.C.P., D.P.H., D.T.M., Professor.
E. D. W. Greig, *C.I.E.*, M.D., D.Sc., F.R.C.P.E., Lt.-Col. I.M.S. (ret.).
George Macdonald, M.D., Ch.B., D.P.H., D.T.M., Major R.A.M.C.
Sir Philip Manson-Bahr, *C.M.G.*, *D.S.O.*, M.D., F.R.C.P., D.T.M. & H.
Oswald Marriott, M.D., B.S., M.R.C.P.
F. Murgatroyd, M.D., F.R.C.P., D.T.M., Lt.-Colonel.
Sir Harold Scott, *K.C.M.G.*, M.D., F.R.C.P., F.R.S.E., D.T.M. & H.
John A. Sinton, *V.C.*, *O.B.E.*, M.D., D.Sc., B.Ch., Lt.-Col. I.M.S. (ret.).
H. E. Shortt, *C.I.E.*, M.B., Ch.B., Lt.-Col. I.M.S.
A. G. H. Smart, *C.M.G.*, M.D., D.P.H., D.T.M. & H.
C. M. Wenyon, *C.M.G.*, *C.B.E.*, M.B., B.S., B.Sc., F.R.S.
F. Norman White, *C.I.E.*, M.D., I.M.S. (ret.).
Vincent B. Wigglesworth, M.D., B.Ch., F.R.S.
Charles Wilcocks, M.D., Ch.B., D.T.M. & H.

Honorary Treasurer: Oswald Marriott.

Honorary Secretaries: C. M. Wenyon and N. Hamilton Fairley.

This concluded the business of the Annual General Meeting.

Transactions of the Royal Society of
Tropical Medicine and Hygiene.
Vol. XXXVI. No. 2. August, 1942.

ORDINARY MEETING

of the Society held at

Manson House, 26, Portland Place, London, W.,

on

Thursday, 23rd July, 1942, at 4.45 p.m.

(After the Annual General Meeting.)

The President,

Sir S. Rickard Christophers, *C.I.E.*, F.R.S., Colonel I.M.S. (ret.),

in the Chair.

PAPERS.

THE TREATMENT OF MALARIA AND SOME POINTS ABOUT THE DRUGS IN USE AGAINST THIS DISEASE

BY

SIR S. RICKARD CHRISTOPHERS, *C.I.E.*, *O.B.E.*, F.R.S., Col. I.M.S. (ret.).

It has been suggested to me that a discussion on the treatment of malaria might be opportune at this meeting and I have been rash enough to contribute a paper on this very large subject. It is obvious that in the time at my disposal it would be impossible to cover in detail all that is embraced under this title. I can only trust that an outline of the broad principles upon which the treatment of malaria, especially at the present time, is based may be helpful to the discussion which I hope may follow.

Our troops are already operating in many malarious countries such as Burma, Madagascar, Irak, Persia and Palestine. The indications are that sooner or later others will be added. It can scarcely be that we shall not see a great deal of malaria. This will no doubt involve extension of our experience in diagnosis and the technique necessary for this. There will be the need to

apply those measures of prevention to which so many have contributed in research and practical application in the last decade or more. But over and above everything, perhaps, is the absolute necessity for treatment of the sick. To-night I shall confine myself entirely to this last-mentioned aspect of malaria.

Like any other disease malaria has symptoms which need to be alleviated, and effects like anaemia which require to be treated on general medical grounds and thus we have "symptomatic" treatment not directly aimed at the cause of the disease but intended to relieve the patient's distress and improve his chances of recovery. Such treatment is by no means unimportant in malaria. Nowadays perhaps we are apt not to give this question of symptomatic treatment quite so much attention as we might. Vomiting, nausea, high temperature and many other distressing symptoms are associated with malaria and it is important to give relief to these in addition to "scotching" the disease which is what now so dominates our mind when treating a case.

However, important as details of symptomatic treatment may be we should not get very far in treatment of malaria if, as let us not forget physicians had once to do, we had to rely on such treatment alone. Nowadays with our powerful specific drugs we have come to look upon malaria as a relatively mild disease. In the absence of these drugs we should see it again in its old grim shape, infections ending ever and anon fatally whilst the physician looked on unable to stop its course, long drawn out perpetually recurring attacks defying all attempts to stop them, profound anaemias and cachexias unamenable to treatment, death from intercurrent disease and altogether a different picture from that most of us now hold. Hence it is that our sheet anchor, our only real hope, in the treatment of malaria is the specific drug and upon its potency we are entirely dependent for any satisfactory result.

Now I think no one will cavil at the statement that up to very recently, the last 10 years or so, the one and only specific drug available for use has been quinine. It is true methylene blue had some effect and even opium and some other preparations have had some curative action ascribed to them. But we should indeed have been in a very bad way if we had to rely on these alone. Probably we shall never know how, or by whom, quinine, or rather cinchona or "the bark" which contains quinine, was first discovered. A very interesting account has recently been given by Mr. A. W. HAGGIS of the Wellcome Research Institution dealing with the introduction of the bark into Europe. It would seem from this that the old story about the Countess del Chinchon must be considered as entirely mythical. Yet the real facts appear to be even more interesting for it seems that the bark that used to be brought originally to Europe for the treatment of intermittent fevers was not cinchona at all, but the bark of a species of balsam and that cinchona bark was used as an adulterant. I do gather, however, from Mr. HAGGIS's paper that cinchona was locally known at Loxa, in the Province of Quito, as a remedy against malaria since it was called the "fever tree." How astonishing, however, that in all the

centuries that quinine has been used no other plant in all the world has been discovered with similar properties !

Cinchona no longer comes mainly, if at all, from the forests of Peru, but as a result of botanical research and pioneer enterprise has been introduced and cultivated on a large scale in other parts of the world, more especially in India and Java. Unfortunately its cultivation, especially that of the heavily quinine bearing forms, has become more and more concentrated in Java so much so that for a long time now this has held a monopoly. With Java now closed to the Allies it is obvious that we must look forward to the supply of quinine for some unknown period being seriously curtailed. So much so must this be the case that it is evident that the supply of a substitute or substitutes must become a most urgent matter.

In this direction one's thoughts go first to the other cinchona alkaloids.

TABLE I.

Some named cinchona and proprietary remedies.

Cinchona febrifuge	Total alkaloids including quinine.
Quinetum	As above, but excluding quinine.
Totaquina	Standardized total alkaloids.
Malarene	Amorphous alkaloids plus nitrophenate, etc.
Malarene (Madras)	Standardized cinchona product.
Quinimax	Quinine with cinchona alkaloids.
Solvochin	Quinine in weak alkaline solution.
Iodoquine	Quinine, adrenaline, iodine and glycerine.
Crinodora (Palusan)	Italian atebrin ?
Paludex	A copper oxyquinoline preparation.
Mapharsan (Mapharside)	Arsenated benzene compound.
M 3	Manganese iodo-mercurate with spleen extract (Italian Biochemical Institute).
Thio-Bismol	Sodium bismuth thioglycollate.
Primaline	Quinacrine, praequine and rhodoquine.

Quinine is not the only alkaloid obtainable from cinchona. Especially in the more robust and low grade quinine yielding species commonly grown in India it may form less than 10 per cent. of the total alkaloids. Cinchona contains four crystallizable alkaloids, *viz.*, quinine, quinidine, cinchonine and cinchonidine as well as amorphous alkaloidal material termed quinoidine. Actually cinchonine was the first alkaloid isolated. Quinine seems to us to have been somewhat arbitrarily selected as the one alkaloid to be used. This choice may, however, have been based on early experiences in treatment. Actually McGilchrist (1915) and others have shown that two of these other alkaloids, cinchonine and quinidine, are about equally effective with quinine, whilst the third, cinchonidine, is also somewhat active. These other alkaloids occur in amounts collectively considerably greater than does quinine itself and they have

been in use, especially in India extracted from the bark along with the quinine as *cinchona febrifuge*, or after the quinine has been removed as *quinetum*. Cinchona febrifuge, according to McGilchrist as shown by samples examined by him, contains about 7 per cent. quinine and 47 per cent. of other crystallizable alkaloids. A product, *totaquina*, of this type guaranteed to a standard content of not less than 70 per cent. crystallizable alkaloids, of which not less than one-fifth must be quinine, was recommended by the League of Nations on the basis that the utilization of these alkaloids would make an effective antimalarial remedy available to a greater proportion of the population than would be the case if quinine alone was utilized.

Besides these naturally occurring alkaloids there are various derivatives of the quinine type, notably *hydroquinine*, a form of compound considered by some authorities to be even more effective than quinine and to be free from some of the undesirable effects of this latter drug. Whether hydroquinine is available in any amount on the market I cannot say, but it is clearly a very suitable form to use especially in cases of quinine idiosyncrasy. Some of the other derivatives are unsuitable owing to their toxic properties, *e.g.*, optochin which has been stated to be liable to cause optic atrophy.

However, it is very doubtful whether even total alkaloids or any quinine derivatives could meet what must now be an enormous demand for efficient substitutes for quinine and for this purpose it is obvious we must turn to recently developed synthetic antimalarials.

Some time about 1924-1926 Schulemann, Schönhöfer and Wingler, working in conjunction with Roehl, synthesized plasmochin, the first antimalarial compound ever to be produced and one of the most outstanding and brilliant feats in chemotherapy which enormously stimulated research in this field. A few years later Meitsch and Mauss (1932) working in collaboration with Kikuth, synthesized a further compound, atebrin, probably the most powerful antimalarial at present known. A number of compounds of the plasmochin and atebrin type were also synthesized by Fourneau and are known as Fourneau compounds. Since then many compounds have been made in different countries more or less closely related to, or identical with, plasmochin and atebrin. As many names at various times have been used in this connection they are apt to be confusing and the accompanying Table II may be useful.

In this country we are most familiar with the original names atebrin and plasmochin. In France there are the closely related compounds respectively of quinacrine and praequine. In Russia the two are acriquine and plasmocide. In this country since the outbreak of the war and manufactured by I.C.I. are respectively mepacrine and pamaquin. Since a great deal of work has been done, notably in this country, on both atebrin and plasmochin under the original names it will probably be less confusing in what follows if I continue to use these names. It seems a pity in fact that it should have been found necessary to change them. Both the recent products are believed to be identical,

TABLE II

Atebrin or atebrin-like compounds.

Atebrin	(first named erion).
Quinacrine	French atebrin. Probably identical with atebrin. First described as 866 RP.
Acriquine	Russian atebrin. Atebrin or closely related compound.
Mepacrine	English atebrin. Believed identical in structure and biological effect with atebrin.

Plasmochin (or Plasmoquine) and plasmochin-like compounds.

Plasmoquine	(first named beprochen).
Praequine	French plasmoquine. Stated to be identical with Fourneau 710 (Rhodoquine).
Plasmocide	Russian plasmoquine.
Pamaquin	English plasmoquine.

Fourneau 915 (Rhodoquine V).

Certuna (Cilional) Effect similar to plasmoquine but much less toxic.

Paludex, Solvochin, Quinimax, etc. } Proprietary preparations.

I understand, respectively with atebrin and plasmochin and FULTON (1937) has shown that biologically mepacrine is indistinguishable from atebrin. The Fourneau compound F.710 (Rhodoquine) is stated to be praequine, or French plasmochin. F.915 (Rhodoquine V) is not so far as I know now much used. Certuna and cilional are two closely related non-toxic compounds having the gametocidal properties of plasmochin.

For all practical purposes therefore we have two well established and now well-known forms of compound in general use as synthetic antimalarials, *viz.*, atebrin and plasmochin.

Plasmochin has the remarkable property of acting upon the sexual or gametocidal forms of the parasite. Attempts to control malaria in communities through this action of plasmochin have, however, not so far met with much success, though it may have a use in reducing the chances of the spread of infection from cases in hospital. Plasmochin also has an action against the asexual forms of the parasites but is rarely used now in treatment of the acute disease and has become more and more employed as a sort of adjuvant to quinine or atebrin, especially in after-treatment and so-called prophylaxis. This is due partly to the fact that it is a rather toxic drug and partly to its not being very effective against the asexual stages of the *falciparum* parasite. It is, however, considered by many authorities to have a beneficial effect in reducing the liability to relapses especially when used in after-treatment or prophylaxis along

with the more effective schizontocidal drugs quinine or atebrin. For this purpose it is given in very small doses, usually not exceeding 0·02 gramme, and then for short periods or well spaced. It is also put up in combination with quinine in the form of plasmochin compound (0·01 gramme plasmochin, 0·125 gramme quinine per tablet) or quinoplasmine (0·01 gramme plasmochin, 0·3 gramme quinine per tablet). It is not considered desirable to administer plasmochin at the same time as atebrin, but it can be given following or alternating with this drug.

The main point for the present is that plasmochin cannot be considered as a satisfactory substitute for quinine in treatment of the acute disease or by itself in after-treatment or prophylaxis. Atebrin, however, as a result of extensive trial all over the world has come to be generally accepted as at least as effective, if not more effective, than quinine. This applies not only to the acute disease, but to reducing liability to relapse and effectiveness in so-called prophylaxis. It is not perhaps a prophylactic in the strict sense of the " causal " prophylactic of JAMES, but then neither quinine nor any other drug known to us can be used in practice for this purpose. From quinine then, should shortage of this drug threaten us, we must pass to atebrin. It behoves us therefore to study very carefully this new effective drug fortunately at our disposal, and come to understand and appreciate both its disadvantages and its advantages, its dosage and methods of administration in a way that was perhaps less important when we had always as a standby an assured supply of quinine. The drug now available in this country, as I have said, is mepacrine, but will be simpler if I continue to speak of atebrin.

All our ideas, however, regarding treatment have been largely built up and based on quinine. It will be desirable, therefore, in the first place to recapitulate very briefly what experience and research has shown to be points in the use of this drug. In the early days in Italy, *e.g.*, in GOLGI's hospital at Pavia, one would see cases in which a single dose of 5 grains or so of quinine was given as a demonstration to students of how one generation of *P. vivax* or *malariae* infection could be completely knocked out if the dose were given at the psychological moment, *i.e.*, in time to catch the sporulation forms. We are not now, as a result of experience, quite so insistent on accuracy in timing our dosage, but generally try to administer the drug over a sufficient period to ensure that there will always be a sufficiency of quinine in the blood through the period of treatment. When in the early days of tropical medicine many cases of supposed malaria seemed resistant to quinine what may be termed the " heroic school " flourished and amounts of quinine which we now consider preposterous were given. Many of these cases were not malaria at all but kala-azar or some other condition altogether : the technique of blood examination had not then been developed as it is now. Largely as a result of the splendid series of researches on quinine treatment by STEPHENS and his collaborators in Liverpool during the last war we now know definitely that these large doses are useless. We do

not now give more than at most 30 grains in a day, *i.e.*, three doses of 10 grains; and many consider 20 grains in the day quite adequate in treatment of acute malaria. Much depends upon the type of parasite and nature of the attack, whether it is the primary attack or a relapse, etc.

Again we know, largely from the Liverpool experiments, that it is no use attempting to eradicate infection by unduly prolonged administration. We may by prolonging treatment cover a period of greatest liability to relapse and so save the patient some attacks, but most authorities now prefer to space administration suitably, usually with an interval or change of treatment. Very commonly about 7 days' treatment is considered suitable to cover the period of the attack and then after an interval perhaps a second period of administration is given, or whatever is considered the most effective after-treatment. There are many modifications used by different authors, including special treatments for chronic malaria which time does not permit me to give in detail. One especially effective form of quinine treatment is the alkaline administration method of SINTON. I shall refer later on to this method in relation to its rationale.

For so-called prophylactic use there are the two common methods: 5 (or 7) grains daily or 15 grains on two successive days in the week. The former is generally thought preferable but it is very difficult to arrive at conclusions in such matters. Most of our information has come from the very comprehensive experiments carried out under the League of Nations and embodied in the *Fourth General Report of the Malaria Commission*. It would appear that 5 grains as a daily dose is rather on the low side and 7 grains is probably better; 10 grains is effective up to a point but is not suitable for long periods. A good deal has to be allowed for conditions, such as fatigue, exposure to the sun, cold, etc., to which the treated person is exposed.

In the main these results, worked out on quinine, are, allowing for the difference in dosage, applicable to atebrin. With atebrin we give not more than 0·3 gramme in the day in three doses of 0·1 gramme. Actually we don't quite know the upper limits here since no one has usually cared to give more than the makers suggest. The toxicity is not apparently great. There is a liability to some gastro-intestinal effects or abdominal pain and, especially apparently in native races, the possibility of nervous or mental symptoms. How far these mental symptoms are encountered in Europeans seems doubtful. More important is the fact that, whilst even quinine may show some cumulative effect, atebrin shows this quite definitely. Hence, not only is the total daily dose pretty well fixed, but the periods for which atebrin is administered must be carefully watched. Usually in the acute attack atebrin is given for a period not exceeding 7 days, and if necessary repeated after an interval. In prophylaxis the chief difference from quinine is in the avoidance of continued daily administration on account of the cumulative effect. Usually the dosage in this case is rather cautious, say 0·2 gramme on two successive days in the week or

even at longer intervals. There are, as with quinine, many modifications of the method employed.

In regard to treatment as a whole the tendency is to combine the various drug treatments. Amy and Boyd (1936), for example, after purging with calomel followed by Epsom salts administer quinine until the initial febrile paroxysms are controlled. Quinine is then stopped and 0·3 gramme atebrin given for 7 days. The patient is then allowed up and given 0·03 gramme plasmochin for a further 5 days. Barrowman (1936), in Malaya, gave 30 grains of quinine for 2 days, and then four tablets (0·4 gramme) atebrin for 4 days. In general, with atebrin the treatment is much as with quinine but one must be more careful.

There are some points about atebrin which it will be well to mention. Atebrin is the bihydrochloride of the alkaloidal base. This is the usual form for oral administration. It is also put up in ampoules as "atebrin musonate" for injection, the so-called musonate being a more soluble salt of the alkaloid and so more suitable for this purpose. Atebrin is a powerful yellow dye and its administration is liable to give rise to yellow coloration of the skin, so that the person taking it may appear to be suffering mildly or severely from jaundice. Actually the coloration is in no way related to jaundice or due to blood destruction and is in no way harmful except aesthetically. I see it is stated that the sclerotics are not affected. It would be interesting to have this confirmed perhaps in the discussion. It is stated, however, that with the smaller doses used in prophylaxis coloration may be absent or negligible. Atebrin can be used in conjunction with plasmochin, but it is not given along with this drug and only in periods preceding it or alternating with it. It is supposed that atebrin does not, like quinine, precipitate blackwater. Cases, however, following atebrin have been described and it is perhaps early to be completely sure on this point. Meanwhile, atebrin is generally preferred where it is thought there is any danger of blackwater. Atebrin has been stated also to be less liable to bring about abortion in pregnancy than quinine. This also cannot be considered very certain since malaria itself is so frequent a cause of abortion. It is generally considered that atebrin should not be used except under medical supervision owing to the possibility of toxic effects. How far this attitude is justified is perhaps debatable, especially when, as may happen, it will very largely have to replace quinine.

So far the points given have been rather to the disadvantage of atebrin as compared with quinine. There is one feature, however, in which atebrin has a very clear advantage over quinine, *viz.*, in its suitability for intramuscular injection. Treatment in the ordinary case of malaria is best carried out by oral administration whether of quinine or atebrin. Should, however, there be any indication of cerebral or other pernicious manifestations parenteral administration is not only a matter of necessity but of urgency. With quinine this must be by intravenous injection and such administration must be carried out with care, either by using a dilute solution or by injecting the drug very slowly. Intramuscular use of quinine in such circumstances is quite unjustified as the

necessary rapid absorption could not be expected. Intramuscular injection of quinine is usually in fact not so much used in pernicious malaria as in treatment of cases of a chronic kind thought to be resistant. Actually the use of intramuscular quinine for such a purpose is largely the perquisite of the doctor babu or low-class practitioner. In the opinion of many it amounts to malpractice. Probably the case treated is not malaria to start with. The results at best form a necrotic mass in the tissues and at the worst lead to tetanus or serious sloughing and other effects. Having in view the now very common condemnation of intramuscular injection of quinine I daresay some of you may have thought it strange that with atebrin it appears to be accepted as a correct procedure, which quite wide use of this method has shown to be. As the reason for this difference in the case of the two drugs is rather interesting I may perhaps very briefly indicate what the explanation appears to be.

The alkaloids of quinine, atebrin and plasmochin are relatively very insoluble and they are usually employed therefore in the form of salts. In the case of quinine there are two types of salts in common use, *viz.*, the hydrochloride (sol. 1-20), the sulphate (sol. 1-800), etc., and the bihydrochloride and bisulphate, etc., which are very much more soluble, say 1 in 1 or 2. For injection the bisulphate or bihydrochloride is usually used as being so soluble. The sulphate of course could not be used, and to give 15 grains of the hydrochloride 20 c.c. of fluid would be required. Atebrin musonate, like the bihydrochloride, would come under the bi- salt type, and so far would not differ from quinine.

Alkaloids are technically " weak " bases and it is characteristic of the salts of weak bases that in solution they " hydrolyse." Hydrolysis consists in the splitting up of a proportion of the salt into the base and " free " acid. The extent to which this occurs depends upon the degree of weakness of the base which is indicated by its dissociation constant, a figure (expressed as pk) which is greater the weaker the base. If the value of the constant is known the extent of hydrolysis taking place at any dilution can be accurately calculated. Quinine has two constants the weakest (that concerned with the bi-salts) being 9·85 and the equivalent acid strength of a 1-2 solution of the bihydrochloride is about 0·3 per cent. hydrochloric acid with a pH of 2. Thus there is no difficulty in understanding why a necrotic slough is formed in the muscle. The weakest constant for atebrin is 6·47 and the hydrolysis with this is negligible. Hence the probable explanation why atebrin does not show the sloughing of the tissues as does quinine on injection.

These constants are most valuable data enabling almost all ordinary reactions of these drugs to be understood or even calculated. One is sometimes asked, for instance, in what state is quinine in the body and it is supposed generally that it must be entirely in the form of the alkaloid. Actually, if one assumes that the pH of the blood and tissues is 7·4, then 88 per cent. of the quinine will be present as the hydrochloride or other mono-salt and only 12 per

cent. as alkaloid. At 7·8 the amount of alkaloid would be 27 per cent. It is possible that with a reduced alkaline reserve alkali administration might raise the pH of the blood, in which case it might be the reason for the effectiveness of SINTON's alkaline method previously referred to. Unfortunately the study of dissociation of these compounds is given little attention and much important and instructive information is thereby lost. (See Tables III, IV and V.)

In atebrin then we have a substitute, and at the present time the only substitute, for quinine. It is a good substitute and we shall probably have to use it extensively. It is a pity its name has been changed to mepacrine, but as they say, a rose under another name would smell as sweet.

That this is the last word in synthetic antimalarials is improbable. Sulphanilamides have not so far given much indication of their usefulness, but SINTON, HUTTON and SHUTE (1939), have produced causal prophylaxis with proseptasine and COGGLESHALL (1938) complete sterilization of *P. knowlesi*

TABLE III.

Calculation of acid strength (hydrolysis) of a salt of a weak base and strong acid in any required dilution.

Example : 5 per cent. quinine bihydrochloride.

Conc. $= 5/40 = 0{\cdot}125$ mol. $\frac{1}{2}\log 0{\cdot}125 = -0{\cdot}031$
pk (pk_2) for quinine $= 9{\cdot}85$. $\frac{1}{2}pk = 4{\cdot}925$.

Formula for hydrolysis :

$$pH = 7 - \tfrac{1}{2}pk. - \tfrac{1}{2}\log.\ \text{Conc.}$$

Substituting

$$pH = 7 - 4{\cdot}925 + 0{\cdot}031$$
$$pH = 2{\cdot}106$$

Equiv. conc. strong acid to above pH

$$= \text{antilog } pH = 0{\cdot}0078 \text{ mol. conc.}$$

Converting to per cent.

$$= 0{\cdot}0078 \times 40 = 0{\cdot}3 \text{ approx.}$$

TABLE IV

Calculation of hydrolysis of 5 per cent. solution of atebrin bihydrochloride.

pk_2 atebrin $= 6{\cdot}47$ Conc. $= 0{\cdot}125$
$\frac{1}{2}pk = 3{\cdot}237$ $\frac{1}{2}\log$ Conc. $= -0{\cdot}031$

$$pH = 7 - 3{\cdot}237 + 0{\cdot}031$$
$$pH = 3{\cdot}796$$

Equiv. Conc. $= 0{\cdot}00016$ per cent. $= 0{\cdot}0064$

$$\text{or } \frac{0{\cdot}3}{0{\cdot}0064} = 47 \text{ times less than quinine.}$$

TABLE V.

Calculation of quinine existing as salt and as alkaloid at 7·4 pH.

Concentration considered $= 0{\cdot}00001$ $\quad$ pk_1 quinine $= 5{\cdot}70$

$pOH = 14{\cdot}06 - 7{\cdot}4 = 6{\cdot}66$ $\quad$ $OH = 0{\cdot}00000022$

$$\log \frac{\text{dissoc.}}{\text{undissoc.}} = pOH - pk_1 = 0{\cdot}96$$

$$\frac{\text{dissoc.}}{\text{undissoc.}} = \text{antilog } 0{\cdot}96 = 9{\cdot}12$$

$$\text{Per cent. dissociated } \frac{9{\cdot}12 \quad 100}{10{\cdot}12} = 90{\cdot}1$$

Total portion conc. dissoc.	0·00000901
Dissociated alkaloid (OH)	0·00000022
Salt	0·00000879
Portion conc. undissoc (alk.)	0·000001
Dissoc. alkaloid (OH)	0·00000022
Alkaloid	0·00000122

$$\text{Proportion } \frac{\text{Salt}}{\text{alkaloid}} = \frac{879}{122} = 7{\cdot}2$$

Percentage salt, 87·8. Percentage alkaloid, 12·2. Percentage similarly worked out at pH 7·8.

Percentage salt, 72·9. Percentage alkaloid, 27·1.

infection by the use of a sulphonamide preparation. Further, GLYNN-HUGHES, LOURIE and YORKE (1938) have, in testing out compounds of the synthalin type, recorded at least one of these, *viz.*, n. undecane 1 : 11 diamidine, effective against malaria, an entirely new type of antimalarial compound.

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THE TREATMENT OF MALARIA IN A HYPERENDEMIC ZONE

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The treatment of malaria in a hyperendemic zone presents several points of contrast from that in an endemic zone. In endemic malaria the parasite is much more frequently *P. vivax*: in hyperendemic malaria it is usually *P. falciparum*. In endemic malaria severe and fatal cases occur at all ages; in hyperendemic malaria the severe cases and all the mortality occur in childhood. In endemic zones the incidence and severity of the disease will be much the same in the native population as among newcomers; in the hyperendemic zone while the native population flourishes, the newcomers are rapidly killed off unless protected against infection or treated for the disease. In the endemic zone infection is comparatively scarce and immunity practically non-existent: in the hyperendemic zone infection is universal and intense during youth but declines towards old age as immunity is established. Hence an epidemic may occur in an endemic zone or among non-immunes when for any reason infection becomes more widespread and intense. An epidemic cannot occur in the native population of a hyperendemic zone. Infection is actually necessary to keep up the immunity. There are no epidemics and no labour problem in West Africa from this cause. On the whole it will be seen that the native population in a hyperendemic zone is very well off compared with those in places where malaria is only endemic. This was postulated by GILL (1921) in a study of epidemic malaria, and demonstrated statistically by GRANVILLE EDGE (1937) in his analysis of Colonial Medical Reports for 1935. The Reports showed less malaria in British West Africa than in the Far Eastern Colonies. The immunity and low mortality depend on hyperendemic infection. This must colour our whole attitude towards prevention and treatment. It has been frequently pointed out that attempts at prevention of infection by anti-larval measures may do more harm than good and even in the treatment of the disease we should try to interfere as little as possible with the development of immunity.

Malaria in Natives

In native adults.—Many observers have commented on the mild course of malaria in natives in the hyperendemic zone. I have found in about twenty recent cases in adult Africans, in which I withheld quinine, that the disease runs a mild course and terminates naturally. The only fatal case of malaria I have ever seen in an African adult has been at a *postmortem* on a pregnant woman. In the vast majority of cases of fever in adult natives—this particularly applies to males—I do not think quinine should be given for the following reasons: (1) Natural recovery will take place without it. (2) It is probably undesirable to interfere with the course of immunity by giving quinine. (3) If quinine becomes scarce then it should be reserved for the severe cases which are seen in children and in immigrants. (4) The giving of quinine to such cases of fever, whether parasites are present or not, confuses the diagnosis. This is most serious in the case of tuberculosis. Here only too often the case is treated as malaria in the early stages when the tuberculous condition is most amenable to treatment. According to Barber (1931) 48·6 per cent. of adults in Southern Nigeria show malaria parasites in their blood. Obviously, therefore, a patient who shows parasites in the blood and who has fever is not necessarily suffering from malaria. This is not merely a theoretical consideration but is of practical importance in a country where the death rate from tuberculosis is higher than that from malaria.

In native children.—The indications for treatment of malaria in native children are difficult to define. Children are in the process of gaining immunity. Between the ages of 1 and 4 the parasite rate may be up to 100 per cent. To treat infection *per se* is unnecessary and undesirable. Even when parasites are accompanied by fever the case may not be malaria. To quote a common instance—a child is brought in suffering from convulsions and fever, with signs in the chest, and parasites in the blood. She may be suffering from cerebral malaria, meningitis or pneumonia, three conditions where diagnosis and treatment must be prompt to be of any use. In spite of the fact that malaria infection is almost universal and does not in itself require treatment there are, of course, cases where urgent treatment is required. The total amount of quinine required to cure the fever in children is very small. It will be found that most cases require injections of quinine.

Treatment of European Cases.

On the whole malaria is a mild disease in the native who is developing a dynamic immunity. Intense and ubiquitous infection is no longer capable of causing an epidemic of disease in such a population. But the potential severity of the disease finds an adequate medium of expression in the adult European. It is not often nowadays we see anything like an epidemic on account of the universal habit of taking quinine. About 18 months

ago, however, an incident occurred which illustrated the potential virulence of hyperendemic malaria. A merchant ship with a crew of fifty-two was directed to the West Coast of Africa. The ship's officers had no previous experience of the coast and did not distribute quinine. After 3 weeks on the West Coast they reached Lagos, where they spent 10 days. Before leaving this port forty-six members of the crew had suffered from fever. On one particular day there were twelve ill on board and eleven in hospital. Of the twelve on board six had temperatures of over 103° and vomiting and had to have injections of quinine. It is in these circumstances, which this incident illustrates, that Europeans live in West Africa and it speaks well for the existing defence measures that morbidity and mortality rates are as low as they are. Immunity could never be allowed to develop in Europeans on account of the virulent course the disease takes in previously uninfected adults. Prevention of infection by anti-larval measures is not practicable. I do not know who first suggested five grains of quinine daily for the perpetual treatment of malaria—we know it is not a prophylactic. Probably it crystallized out as a tradition from the days of the early pioneers. It has been subject to much criticism but in a modified form it has stood the test of time. It has many drawbacks. For example, the dose for each individual has to be adjusted—often by trial and error. It does not prevent infection and it is inadequate once fever occurs. At the same time it so reduces the parasite content of the blood that it often confuses diagnosis. On account of this it is the indirect cause of many cases of low fever and so-called tropical neurasthenia. Probably some cases of mental deterioration and alcoholism could be traced to it too. It interferes with immunity and it intrudes on the diagnosis and treatment not only of malaria but of every febrile condition which affects the European in the hyperendemic zone.

Standard Treatment. In the past the standard treatment for fever in Nigeria was—20 grains a day while the fever lasted, 15 grains for a few days, 10 grains for perhaps a week and then back to the routine 5 grains. There has recently sprung up what might be described as a new school of thought were it not that it has reverted to an older method of heroic dosage. For the acute fever they recommend 30 grains of quinine daily by mouth plus 10 grains intravenously with four tablets of atebrin. The maintenance dose is 15 grains of quinine and one tablet of atebrin daily. The fact is that it would be difficult to devise a régime anywhere between these two extremes which did not produce good results in most cases. It must be remembered in this connection that the vast majority of attacks of fever are treated not by doctors but by the patient and his friends and that the results are generally fairly good. The low dosage popular in Nigeria is accounted for partly by the fear of blackwater. It is an irrational prejudice, however, because 30 grains of quinine will not produce blackwater in a patient who can take 20 grains with impunity. On the other hand, the heroic régime is unnecessary in most cases. There may be some-

thing to be said for the large maintenance dose in certain emergencies—for example, a civilian on a long trek or a soldier actually in the battle zone. For my own part I prefer the usual textbook régime of 30 grains daily for the acute stage and the maintenance dose of about 5 grains.

Practical Points in Treatment.

Possibly the following practical points in treatment may be useful. Injections of quinine are frequently required. Our Chairman has condemned intramuscular injections, but intramuscular quinine has advantages. It is convenient. It can be used for patients treated at home or on board ship, for example, where intravenous therapy would be difficult. It is practically indispensable in infants and children. It is effective and indeed the results are often dramatic.

Intravenous quinine has the advantage of being more or less painless and there is no sloughing of the tissues. There are, however, no empirical findings to show that it is superior to intramuscular quinine. The purely theoretical advantage that it brings the drug rapidly into contact with the parasite appears in many publications. But malaria parasites have been known to flourish, in a concentration of quinine stronger than that achieved by an intravenous injection. That is not to say, however, that it is ineffective. It is just that *a priori* reasoning is notoriously unreliable in malaria. Indeed, it might easily have deprived us of the use of quinine altogether.

Intravenous quinine must stand or fall by empirical findings. Is it superior in the average severe case? I do not think there is anything to choose. Is it superior in the comatose case? This is the really crucial point and we have not enough data to decide on the issue. I only know that intramuscular quinine, plus saline and glucose intravenously, is remarkably successful.

Finally in the matter of abuses. Intravenous quinine will be subject to the same abuse and possibly with more dangerous results. I know this from experience. It is the inferior type of practitioner who gets abscesses after intramuscular injection. Are we going to encourage this individual to inject his quinine through the anterior fontanelle in the case of infants?

The position is that we have two excellent remedies both of which require some technical skill. From the empirical standpoint we have not sufficient data to decide yet which is the better. It is, unfortunately, only to be expected that the one requiring the least skill is subject to the most abuse, but surely it is a counsel of despair to condemn it for that reason.

Fever should be treated promptly. A certain publication intended for lay consumption advocates waiting for the fever to subside before giving quinine. This has led to fatalities on board ships. This is another instance which shows that observations from artificial malaria and endemic malaria—mainly benign tertian—must not be applied directly in hyperendemic conditions. If quinine is not given quickly, incessant vomiting and coma may develop.

Headache is a common symptom when there is high fever and some residual headache may remain for anything up to 3 months in cerebral cases.

Purgatives are not necessary in the acute stage and I think they are overdone. They cause coating of the tongue and abdominal distension by hurrying on the undigested food to ferment in the large bowel, and it is most distressing for the patient to have a bowel movement when he is seriously ill. However, the nursing sister considers the giving of purgatives her own special prerogative, and there is not much hope of reform in this generation.

After-Treatment.—Questions are often asked about the after-treatment of malaria when the subject leaves the hyperendemic zone. The general rule hitherto has been to recommend 5 grains quinine daily for 2 months after leaving the West Coast. Although fairly successful on the whole, there have been many failures and blackwater fever has occurred quite frequently. I have recently advised Europeans to take a course of atebrin and quinine beginning 10 days after leaving the Coast. The course consists of two tablets of atebrin and two 5-grain tablets of quinine daily for 7 days. It is too soon yet to recommend it generally, but the results so far in about fifteen cases have been successful. The régime was designed to affect the very latest infection the subject received on the night before he left the Coast, and to substitute a short treatment for a long one. Most people will not take 5 grains daily for 2 months especially as home leave is so short. Another problem concerns strangers who visit the Coast for a very short period. Should they take quinine? It depends on the length of stay, the localities they visit and the season of the year. If the probabilities are in favour of infection I advise a maintenance dose of quinine while they stay and after-treatment when they leave.

Blackwater Fever.—A practical point in connection with the treatment of blackwater fever is that the causes of death in this disease are anuria, hyperpyrexia and anaemia with the consequences. Of these the least frequent is anaemia. Many successes have been reported recently from blood transfusion. In these reports one can always see that the anuria is treated as well by intensive alkali and water therapy. Unfortunately too much attention is focused on the transfusion with the result that intensive alkali therapy—which has a good reputation—is not practised as often as it should be. Alkali therapy is a *sine qua non* whether blood is transfused or not. It is available in circumstances when blood transfusion is impossible. Blood transfusion was intended to be an adjunct to alkaline therapy—not to replace it. The urine can and should be rendered alkaline within 24 hours; I consider this is an ample margin to allow.

Low Fever.—Low fever is a condition which requires careful diagnosis. I once had a female patient who complained of low fever because her thermometer registered 97·4° every evening! There are, however, genuine cases which run a temperature of 99-100° F. for weeks at a time. Probably the most frequent causes are malaria and pyelitis. There are, of course, many other

causes. The diagnosis of the malaria cases may prove difficult on account of the 5 grains of quinine daily. If quinine is discontinued for 4 or 5 days repeated examination of the blood will usually settle the diagnosis. Some people consider this rather dangerous. However, if a bout of fever with rigors does occur in such cases it is not such a bad thing. After adequate treatment and convalescence the patient usually feels much better and may get rid of certain neurasthenic symptoms associated with the low fever.

Maintenance Dose.—As already mentioned, the maintenance dose of quinine or atebrin must be adjusted to meet individual requirements. Five grains of quinine daily suits most people. Two tablets of atebrin on Friday and Saturday are becoming popular. It is about equal to 5 grains of quinine. There is still, of course, much speculation about the exact dosage necessary. I believe that the ideal dosage can only be determined by the individual himself. In this connection I should like to refer to the treatment of troops in a hyperendemic zone. WENYON (1921), in his survey of malaria in Macedonia during the last war, emphasised the impossibility of preventing malaria by anti-larval measures in the fighting zone. The last war was mainly static whilst this one is mobile. Anti-larval measures are, therefore, out of the question. It might be urged that under camp conditions troops might be protected from infection. Actually such protection is almost impossible to achieve. In the remote event of it being successful it might promote a feeling of false security. The time spent in camp is comparable to the first tour of the civilian in the hyperendemic zone. This is the period during which he learns how to control his malaria by adjusting the dose of quinine. If the civilian neglects his quinine he very soon learns from his mistake in the unforgiving environment of intense infection. A certain European—an unsociable crank who never went out at night—managed to spend 9 months in a screened house in Lagos without taking quinine and without getting fever. His example was responsible for malaria in more than a dozen of his comrades. In a well-protected camp a soldier might similarly get away with it and infect his comrades with his bad example. This could only lead to disaster when the troops moved up to the fighting zone. I would recommend nothing beyond a mosquito-net and maintenance dose of quinine—just as in the case of the civilian.

SUMMARY.

I have tried to take a realistic view of the problem of malaria in a hyperendemic zone and to show how it differs from endemic malaria. In hyperendemic conditions anti-larval measures could never solve this problem for the native and are probably undesirable. We should not interfere with infection for it is on this the evolution of immunity depends. In this respect the interests of the native might appear to clash with those of the immigrant but in reality it is not so for various reasons which we need not consider here. Future advances in the handling of hyperendemic malaria will probably be along the lines of

improved technique in individual treatment. For the native, immunological considerations must determine the technique. In the case of the European, we must rely on continual treatment of a disease, which we cannot hope to prevent by sanitation or control by immunity.

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DISCUSSION.

Professor Warrington Yorke : I have listened to Sir RICKARD CHRISTOPHERS' address, as I am sure everybody has, with much appreciation. With most of it I am in entire agreement, but with one point, to which I shall refer later, I find myself in complete disagreement.

Two facts seem to me to be of overriding importance at the present time: firstly, that we have lost Java and with it over 90 per cent. of the world's supply of quinine, and secondly, that our troops are already operating in many malarious countries and that many of our ships are calling at malarious ports. It seems obvious, therefore, that the demand for anti-malarial drugs will increase enormously. This is, of course, a very serious situation, demanding immediate action in two directions: firstly, the manufacture of an adequate quantity of the only efficient synthetic substitute for quinine, viz., mepacrine, and secondly, the conservation of the existing stocks of quinine. The first, although obviously of the greatest importance, is a responsibility which we must leave to others. The second is, however, a matter which in some of its more important aspects touches this Society very closely, because the great bulk of the world's production of quinine is used for the treatment of malaria, and the only material way in which we can conserve the stocks of quinine is by using it as economically and efficiently as possible in the treatment of this disease.

It is a remarkable fact that although everyone is agreed about the value of quinine and millions of cases of malaria have been treated with it, there is no unanimity of opinion regarding the way in which it can best be used. One of the greatest virtues of quinine is its extremely low toxicity, and this fact has undoubtedly tended to encourage the use of unnecessarily large doses. You, Sir, have referred to the activities of the " heroic school " and to the restraining influences of the Liverpool researches in the last war. In this work we attempted to answer the following questions :—

What is the smallest amount of quinine necessary to control the acute attack ?

Is it possible with massive doses of quinine to sterilize a malaria infection within a few days and, if not, can the infection be sterilized by a prolonged course of quinine treatment?

With the object of obtaining an answer to the first of these questions, groups of patients suffering from acute simple tertian malaria were treated with quinine in doses varying from 5 grains to 120 grains on each of two consecutive days only. Even in the group treated with the smallest amount of quinine, *i.e.*, 5 grains on each of 2 consecutive days, parasites disappeared from the peripheral blood and the temperature became normal in over 80 per cent. of the cases, and this always happened when the doses were 10 grains or more. Within a few weeks, however, the majority of cases in all the groups had relapsed —even those given such large doses as 90 grains or 120 grains, which are beyond the limit which can be tolerated by most ordinary individuals.

These results suggested that in the treatment of acute simple tertian malaria nothing was to be gained by giving more than 20 grains or 30 grains of quinine for a couple of days, and that it was impossible to produce a *sterilisans magna* by pushing the dosage to a degree which was definitely toxic. A series of cases of acute malignant tertian malaria were given a similar 2-day treatment of 30 grains of quinine, and here again in all instances the temperature became normal and trophozoites disappeared from the blood.

With the object of answering the second question, *i.e.*, whether a definite cure can be obtained by a long course of large doses of quinine, groups of cases of simple tertian malaria were given orally daily doses of up to 45 grains for as long as 8 weeks, and others were given the same doses on 2 consecutive days only for the same period. Although the infections were usually controlled whilst the patients were taking these long courses, many relapsed within a few weeks of the end of treatment. The general conclusions reached from this part of the work are firstly, that many cases of simple tertian malaria are not cured by large doses of quinine administered daily or twice weekly over a period of 8 weeks, and secondly, that given a total weekly dose of quinine, it is better, from the palliative or suppressive point of view, to divide it into two equal parts and administer one on each of two consecutive days, than to divide it into seven equal parts and administer one on each of 7 consecutive days—in other words, as a suppressive treatment *interrupted* is preferable to *continuous* quinine treatment in simple tertian malaria.

It is upon these observations that the method of quinine treatment of malaria, which has been followed in Liverpool for many years with satisfactory results, has been devised. It consists in controlling the acute attack by the oral administration of 30 grains of quinine sulphate in solution for 4 consecutive days: the patient is then discharged and instructed to take 20 grains of quinine every Saturday and Sunday for the next 8 weeks, and if he relapses after that, to continue the week-end treatment for a further period. It is very possible that the dosage of quinine involved in this scheme of treatment is unnecessarily large, but as the drug is relatively harmless and as until recently it was

plentiful, it seemed wise to err on the liberal side especially in the case of individuals like seamen who may be cut off from medical assistance for prolonged periods.

Owing, however, to the loss of Java, the quinine position has completely changed and it is now necessary to take every step possible to reduce consumption. Regarding the imperative necessity of controlling the acute attack, especially in an unacclimatized population, there can be no doubt. Sir RICKARD CHRISTOPHERS has referred to the appalling state of affairs which would result from failure to do this. The amount of quinine used for prophylactic or suppressive purposes is probably much greater than that used for treatment of acute attacks, and I think there can be little doubt that much of it is wasted. In fact, it might well be argued that better results would be obtained from simply treating the acute attacks as they occur. I do not mean to suggest that prophylactic or suppressive treatment with quinine should be entirely abandoned, but that it is now necessary to employ the drug for these purposes much more carefully than hitherto. Obviously suppressive treatment is necessary for troops campaigning in malarious countries and for seamen voyaging to malarious ports, but we must keep clearly in mind that treatment of the acute attack is essential, and that if there is any shortage of antimalarials it is the suppressive form of treatment, at present involving the consumption of such vast quantities of quinine, which must be drastically reduced.

Although, therefore, if we are to conserve quinine, the bulk of the saving must come from suppressive treatment, it is important that we should not waste the drug in the treatment of the acute attack. To illustrate my point, may I refer to an example of treatment of a case of malaria by quinine suggested by the Army medical authorities. It is as follows: 30 grains daily for 6 days, 20 grains daily for 4 days, interval of 7 days, and finally 20 grains daily for 7 days. The total amount of quinine involved in this course is 400 grains. Now I cannot help asking myself what this course is intended to do. If it is designed simply to treat the acute attack, probably at least three-quarters of the quinine is wasted, and if it is intended to sterilize the infection, it will fail. I have referred to this scheme of treatment, which is representative of many similar methods, because much quinine could be saved if it were abandoned.

Before I leave the subject of quinine, I must mention one other matter. It was with surprise and consternation that I listened to Sir RICKARD's scathing denunciation of the intermuscular use of quinine. I entirely agree that the habitual and repeated administration of intramuscular injections of quinine in chronic malaria is simply a money-making device of unscrupulous individuals, but I part company when you say, Sir, that the method is unjustified in serious cases with pernicious manifestations because the necessary rapid absorption could not be expected. I have found one or two intramuscular injections of quinine bihydrochloride invaluable in the treatment of really serious cases in which it is urgently necessary to make certain that the patient will absorb some of the drug quickly. I am well aware that only a small portion of the drug is

absorbed because I have found considerable quantities in the muscles of experimental animals weeks after the injection was made, but the important fact remains that sufficient is absorbed to produce the desired results. In Liverpool, where we must have given very many hundreds of intramuscular injections during the last 25 years, we have never produced the dire results referred to by you, Sir, and I am confident we have saved many lives. I was so surprised by your condemnation of this procedure that I took the trouble to look up the Liverpool investigations to which you refer in such complimentary terms, and I found that in this series of papers records are given of twenty cases of simple tertian malaria and thirty of malignant tertian malaria treated with intramuscular injections of 15 grains of quinine bihydrochloride on each of 2 consecutive days. The results were excellent: in all cases parasites disappeared from the blood and the temperature became normal within 1 to 2 days.

I have no time to refer to mepacrine. It is, at the present time, the only real substitute for quinine, and it is of the utmost importance that steps be immediately taken to manufacture a sufficient quantity to meet all requirements. The stocks of quinine are limited and the lack of sufficient antimalarial drugs in times like these would be attended with consequences too dreadful to contemplate.

Before I close there is one other matter to which I should like to draw your attention and upon which I should be grateful for information. I can illustrate the point best by referring to the so-called Standard Army Treatment of Malaria, which runs as follows:—

Days	1	to	2	quinine 30 grains daily.
	3	„	7	mepacrine 0·3 gramme daily.
	8	„	9	no treatment.
	10	„	14	pamaquin 0·03 gramme daily.

The point which particularly interests me—and it is not unimportant because the drug is difficult to manufacture—is, what is the pamaquin supposed to do?

I presume the idea is that it lowers the relapse rate. But what is the evidence? As SINTON's name is frequently mentioned in this connection I took the trouble the other day to look up the work of SINTON and his collaborators which was published in 1928 and 1929. They treated groups of cases of simple tertian malaria with quinine alone, 30 grains daily for 3 weeks, with pamaquin alone, 0·08 gramme daily for 17 or 28 days, and with various mixtures of quinine and pamaquin daily for 3 weeks. It was found that the number of relapses occurring in the groups treated with pamaquin 0·06 gm.+quinine 20 grains or pamaquin 0·04 gramme+quinine 20 grains daily for 3 weeks was much less than that in the control group treated with quinine 30 grains daily for 3 weeks.

Shortly after the publication of this work, this plan of combined treatment

with pamaquin and quinine was tried in India on a wholesale scale amongst British and Indian troops infected with simple tertian malaria, and MANIFOLD (1931) recorded the results of treating over 3,000 cases by SINTON's method of pamaquin 0·04 gramme+quinine 20 grains daily for 3 weeks; his results entirely confirm those of SINTON. There is thus strong evidence that this 3 weeks' course of pamaquin+quinine does, in fact, reduce the relapse rate materially in simple tertian malaria. In passing it should, however, be noted that SINTON records that 64 per cent. of the patients receiving the 0·06 gramme dose of pamaquin showed signs of toxaemia and 25 per cent. of those receiving the 0·04 gramme dose. In MANIFOLD's series of 3,000 cases treated with the smaller dose, 21 per cent. of the British cases and 10 per cent. of the Indian cases exhibited signs of toxaemia.

It is generally agreed that pamaquin has no therapeutic action in malignant tertian malaria, and it is therefore not surprising to find that SINTON records that relapses occurred in ten of the fourteen cases of this disease treated with pamaquin alone or with pamaquin+quinine. AMIES (1930), writing from Malaya, stated that the results obtained did not support the view that pamaquin intensifies the action of quinine on the asexual forms of *P. falciparum*; and GREEN (1934), also referring to Malaya, reported that later results regarding the reduction in the relapse rate in simple tertian malaria by the combined pamaquin +quinine treatment were less encouraging than the earlier records, and he goes on to stress that as pamaquin has no effect on the ring forms of malignant tertian malaria, the combination of the drug with quinine can hardly be expected to reduce the relapse rate in this disease. AMY and BOYD (1936) give interesting results obtained in the treatment of large series of simple tertian cases in India with atebrin+pamaquin, quinine 20 grains+pamaquin 0·03 gm. daily for 14 days and also for 21 days, and other forms of treatment. The relapses were as follows: atebrin+pamaquin 11·2 per cent., pamaquin+quinine daily for 14 days 10·2 per cent., pamaquin+quinine daily for 31 days 13·6 per cent., and other forms of treatment 27·1 per cent. Here, again, we have support for SINTON's contention that pamaquin+quinine daily for 3 weeks reduces the relapse rate in simple tertian malaria. AMY and BOYD's figures for malignant tertian malaria showed that the relapse rate after atebrin+pamaquin and pamaquin+quinine was about the same as that following other forms of treatment.

But what has all this to do with the Standard Army Treatment of Malaria? Clearly it bears no resemblance to SINTON's combined pamaquin-quinine treatment, and I should be very grateful if somebody would tell us whether there is actually any evidence that it does reduce the relapse rate in either simple tertian or malignant tertian malaria and, if not, what does it do that the quinine or the atebrin alone would fail to do.

Major T. Rowland Hill: I should like to make a few remarks confined to the treatment of subtertian malaria and with particular reference to those

regions where it is hyperendemic and where history has shown it to be, as recently as in the war of 1914-18, a major, and often the most important, factor in a campaign. As the official history of the medical services in the last war pointed out, malaria was by far the commonest of all diseases affecting the Forces. This official history made another important though fatalistic observation, that it was inevitable that a force operating in a malarious zone must show a high malarial incidence whatever measures were taken, and that the policy to follow must therefore necessarily be to keep troops for as short a time as possible in the affected area. Circumstances may make it impossible to do this, and European troops may have to reside and fight in hyperendemic zones for prolonged periods. The pessimistic view just quoted may have been true at the time of the last war, but is it so today? This is a most important question to answer. As did Sir RICKARD CHRISTOPHERS, I will limit my remarks to the treatment of malaria in the individual patient. I feel sure from personal observation in hyperendemic equatorial areas that the answer to the question just asked is that the pessimistic view of 20 years ago need not now be held without considerable modification. With the aid of the synthetic drugs in addition to quinine it should be possible greatly to lower the incidence of clinical subtertian malaria among Europeans, including large numbers of troops in affected regions. One hears much about the shortage of drugs and the necessity of economy and warnings against using too much because of bad effects on the patient. Memories are so short that one needs to recall *how uncontrollable some epidemics of subtertian malaria were in the last war.*

In my opinion it is possible to pick holes in the methods of treating subtertian malaria that were popular and standard in some tropical areas before the outbreak of this war. Two factors seem to have made the treatment of many cases less adequate than it could have been. The first was the fatalistic view that malaria was inevitable in almost everybody; so why overtire yourself treating it with unnecessary energy? Just apply enough treatment to give symptomatic relief to a patient during an attack and then let him go until his next, usually not long deferred, bout. No wonder that in certain regions a tour of service of 18 months was considered the longest that an average European could enjoy. The second factor was perhaps the result of a swing of the pendulum from the old days of drenching patients with excessive quantities of quinine to the practice of giving small amounts and of being reluctant to increase them, the result being the undertreatment of many cases. *In my view under-treatment is the greatest fault in the present-day management of cases of subtertian malaria in the hyperendemic zones themselves.*

It is of course easy to bring the temperature down in an attack of subtertian fever in a few days, in most cases, with oral quinine, *e.g.*, 30 grains a day for 5 days. In a very high proportion of such

cases, however, clinical recrudescence occurs a week or fortnight or so afterwards and those working in affected areas are familiar with numerous cases of this kind, which give a history of many recrudescences at short intervals with subsequent severe anaemia and invalidism. It is most important not to forget that, in this form of malaria, exposure to strain and fatigue greatly increases the predisposition to recrudescences, a point sometimes overlooked in civilian practice in the affected regions where the majority of European civilians lead comparatively easy lives under good conditions. The effect of strain in evoking clinical manifestations was an important factor in the outbreaks of the disease which were so serious in tropical Africa in the last war. It can be assumed that in hyperendemic areas 100 per cent. of the Europeans are infected and in much of tropical Africa they take a regular dose of a prophylactic anti-malarial drug. The common practice, which has not altered in 40 years, is that of 5 grains of quinine a day. This appears enough to keep a fair proportion of civilians living under good conditions well and efficient enough to last out their 18 months tours. It does not prevent a considerable incidence of subtertian malaria among them. For those exposed to excessive strain it is, on the other hand, totally inadequate. A considerably bigger dose, 15 grains a day over a prolonged period will not always prevent clinical attacks. Life in hyperendemic regions has shown that regular prophylactic dosage is necessary, and also that quinine is imperfect for this purpose especially under conditions lowering individual resistance. Personal experience, supported by much published observation from different parts of the world, indicates that atebrin is much superior to quinine as a clinical prophylactic. The standard civilian dosage under good conditions is 0·4 gramme of atebrin a week, this being much better than 5 grains of quinine a day. In this regard the work of JUNGE in Liberia is worth mentioning. Again under conditions of strain, as in war, this dosage is probably inadequate. It is the minimum amount giving satisfactory results in civilians in peace time, and therefore is almost certainly insufficient under more exacting conditions.

It is not the toxicity of the drug which prevents people from taking more but its tendency temporarily to turn people somewhat yellow. Incidentally, the sclerotics are turned as yellow as anywhere else, the statement that they are spared being misleading. Much has been said of the toxicity of atebrin, most of it being without foundation. Experience shows it to be as non-toxic as any drug can be expected to be. In the treatment of many hundreds of cases, many with fairly heavy dosage, I never saw a single case of mental excitement, of gastro-intestinal symptoms or of any toxic response to the drug whatever. Yellowing of the skin may be a horrifying prospect to womenfolk fighting a losing battle with their complexions. It is clearly of no consequence among troops in war time. My own view, based on my experience, is that, under conditions of strain, 0·1 gramme a day of atebrin is a most effective clinical prophylactic and should be standardized. I feel sure that this dosage would produce a great

lightening of the malarial problem and that it would modify the pessimistic official view of the last war. I have never seen anyone who had become perceptibly yellow from atebrin ever develop any attack of malaria, and I doubt if an attack could occur in such a person. In fact I think that if it is desired to give a body of men as much passive immunity to clinical malaria as possible an attempt should be made *to try and get and keep a slight yellow tinge in their skin with atebrin*, disregarding the slings and arrows of those self-complacent conservatives who delight to detect somebody who is taking atebrin as a prophylactic, point their fingers at him and chuckle " Chinaman ! " I have heard an unofficial report that the German troops sent into the Struma valley in this war were dosed for a considerable period beforehand with atebrin.

If what we have said is true, then by superior clinical prophylaxis in affected areas one belligerent in tropical warfare might gain a considerable advantage over the other, which is one reason why today's discussion is so apposite. As far as treatment is concerned, the guiding principle should be to terminate the attack completely as quickly as possible. Therefore, though treatment need not be greatly prolonged, it should be intensive at the onset. Haemological examination shows that in one paroxysm many thousands of red cells may be destroyed. Quinine acts more quickly than atebrin when given by mouth, but patients treated with atebrin show a more complete return to full health than those treated with quinine. I agree with Professor YORKE in his criticisms of the long-drawn-out treatment of acute attacks. There should be intensive treatment not to destroy infection, which is impossible, but to knock out the clinical attack very quickly. This only applies to subtertian malaria. I think the treatment should be intensive for the first two or three days, and that can be done with very good results by combining atebrin with quinine by mouth. I do not think it is sufficiently recognized that the two can be given in the ordinary full doses together without the least harm. Ten grains of quinine three times a day for 7 days and 0·01 gramme of atebrin three times a day simultaneously produces excellent results in a large number of cases in troops. The treatment of the acute attack of subtertian malaria should be intensive treatment of the attack followed by prolonged " anti-relapse " treatment. I am sure that as an anti-relapse drug atebrin is far more satisfactory than quinine. Something between four tablets and seven tablets a week, 0·4 to 0·7 gramme atebrin, will give excellent results in preventing recrudescence and further destruction of red cells, anaemia and invalidism.

I must refer before I close to the bogy of too much quinine or too much atebrin. Particularly this applies to intravenous quinine. I myself have given now between 300 and 400 injections of intravenous quinine, 10 grains at a time, without seeing a single case of toxic effects. Intravenous is more effective than intramuscular quinine and the latter should never be given. I think so many unnecessary warnings have been uttered against intravenous quinine that the pendulum has swung dangerously far and its use is often withheld followed

by unnecessary loss of life. Medical officers should be much more ready to use it in cases of subtertian malaria in the field. On the lines I have described it should be possible greatly to reduce malaria among troops in war and to falsify the gloomy predictions of the last war.

Sir Philip Manson-Bahr: I agree with the last speaker in a great deal that he has said. I happened to be one of the first to work with the synthetic drugs in malaria a good many years ago, and the conclusions I came to were published in the *Proceedings of the Royal Society of Medicine.* Subsequent investigations have never fundamentally shaken me in my beliefs. I believe with the last speaker that quinine and atebrin go well together, in the doses advocated, in the treatment of subtertian malaria; but I cannot back you up, Sir, in claiming that intramuscular atebrin is as effective as quinine; nor that intravenous injection is either. There have been several cases of cerebral malaria in London. People who have come from South Africa and have stopped at Freetown on the way. I have been called in when the patient was in coma and found he had had intravenous atebrin. I examined the blood and found that large numbers of parasites were still present. I gave him one injection of intravenous quinine, 10 grains, and within 2 hours he had regained consciousness and he got perfectly well.

The President: Did you give the atebrin?

Dr. Manson-Bahr: No, his doctor gave him atebrin musonate. I saw the ampoule and there was no question that the drug had been given. If you say that intramuscular atebrin musonate is entirely harmless, I do not agree. I have seen large abscesses as the result: There has been a lot of loose talk about intramuscular quinine. I have always said and maintain now that one or two injections work miracles in very serious cases but you don't want to make your patients' buttocks into pin-cushions. In the great majority of postmortems after intramuscular quinine injections death is found to have resulted from sepsis. The patients were not dying of malaria. I am as perfectly convinced of that now as I was 24 years ago. As regards atebrin staining, I entirely agree with the last speaker that if you get pigmentation a relapse of subtertian malaria does not occur. Also I agree with what he said about sclerotics. It is extraordinary, although this possibility has been much advertised, how few people realise what has happened. Once I had a young Indian brought to me to know whether he was suffering from tuberculosis or malaria. I had no difficulty in finding that he had got malaria, and I advised a quinine-atebrin course, which was successful. Two years afterwards he got a fever at his school, and was put by his guardian on a course of atebrin which he took for 4 weeks. He recovered and went to Switzerland, and while there became yellow. He was admitted to a clinic and all sorts of investigations were undertaken to find the cause of the supposed jaundice, and I was severely criticized by his family for prescribing in the first instance a drug which could give rise to icterus.

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COMMUNICATIONS.

SUBTERTIAN MALARIA IN WAR.

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INTRODUCTION.

The problem of malaria amongst troops operating in the face of the enemy in endemic regions has been amply demonstrated by history to be much more acute, serious and difficult of solution than that of the disease as it affects, in the same regions, either troops under garrison conditions or civilians. It has been shown in the past, at heavy cost, that this important difference has not always been fully appreciated.

Grave failures have followed attempts to overcome the malarial onslaughts on a fighting army by using those comparatively light and brief forms of treatment which have been found tolerable among a civilian population unexposed to the rigours of war.

History.

A study of the past reveals that outbreaks of malaria in an army may influence victory or defeat and that the disease may become the biggest factor in a campaign. Sir RICKARD CHRISTOPHERS has said "Malaria in the past has influenced the results of wars; the epidemiology in armies in the field may have special characteristics and the prevention and treatment of malaria in such conditions have special features." Also MANSON-BAHR that the "malignancy of subtertian malaria in military service is due to the exigencies of war" and the Army Manual of Hygiene that "malaria may become the outstanding disease in any area where climatic conditions are suitable and may overshadow all other considerations in the success or failure of a campaign, as it did with the Salonika Force during the Great War."

The last war proved outbreaks of subtertian malaria to be the most grave form of the disease, bringing a persistent morbidity and a high mortality. This form of the disease is, of course, widespread, intense and perennial in the tropics, and it is to tropical warfare as distinct from that in the subtropics that this article is chiefly devoted.

However, serious outbreaks in the last war occurred as far north as 40° latitude. Macedonia produced 30,000 cases involving 25 per cent. of the force. The 1918 Persian expedition suffered heavily with many deaths. From the 50,000 tropical East African troops, two years of war, 1916 and 1917, saw 120,000 admissions to hospital with malaria. A vast quantity of human wastage was produced with much unavoidable invaliding. A comment from the official history of the R.A.S.C. on this campaign is illuminating: "The country was unhealthy and white drivers quickly became victims to malaria. Reinforcement personnel were reckoned as useless for some six weeks after arrival, until they had had their first bout of fever. When they did get down to work their times between admissions to hospital were very short." They cannot have been much use even after their "first bout of fever"!

Following the advance into the subtertian area in the Jordan Valley in 1918 the already high malarial incidence rose enormously with 773 deaths.

Little profit is to be gained from recalling similar experiences in wars previous to the last one except that the Ashanti expedition of 1896 may be mentioned. During this brief campaign of 6 months there were 1,401 admissions to hospital with subtertian malaria out of a force of 5,213. Amongst the admissions were 40 per cent. of the officers.

Some Features of the Disease.

It is most serious amongst troops on the march. Military authorities have to avoid judging its danger by its incidence under garrison conditions; if they made the mistake of doing so they might be caught unawares when conflict came. Conflict is liable to bring with it a tremendous rise in incidence which,

as experience has shown, may be difficult to control. As WENYON remarked of Macedonia, " It is doubtful if any appreciable reduction in infection took place during our stay in the country."

Subtertian malaria tends to be prostrating. Anaemia from haemolysis and depressed haematopoiesis is almost constant and often appears rapidly.

Briefly spaced recrudescences in lightly treated cases are frequent, together with intractable states of chronic debility. The insidious form is liable to be particularly common among troops if care is not taken. One short attack, for which he may give himself a day of quinine without reporting sick, is quickly followed by another until the soldier has steadily descended to a debilitated condition. A few examples may be given.

Case 1.

Three weeks after entering an endemic zone a man developed an attack of subtertian malaria in spite of having taken 5 grains prophylactic quinine daily. He remained in bed only 2 days and the attack was followed by five recurrences at fortnightly intervals. Increased quinine was given during each attack. After 4 months parasites were still found in the blood. He was anaemic, easily exhausted, unfit for work and cachectic.

Case 2.

During the first 8 months of his residence in the tropics this individual occasionally " felt hot " and took extra quinine for a day in addition to the 5 grains prophylactic dose which he always took as a daily routine. During the last 2 months he felt severe exhaustion, dyspnoea on exertion and grew thin and pale. Two months after his arrival parasites were still found. Hb percentage was then 50, red cells 3,000,000 and splenomegaly marked.

Case 3.

This patient had lived in a tropical endemic zone for 3 years. During the first two of these he experienced ten attacks of the disease and these gradually became more frequent until he was sometimes ill for 2 or 3 months continually. Eventually he reached a state of invalidism with much fatigability, inability to work, anaemia and splenomegaly.

Case 4.

Ten days after landing in the tropics this patient had his first attack of subtertian malaria. He received 5 days' treatment of 25 grains quinine a day, then 7 days of 10 grains, and thence onwards 5 grains prophylactic quinine daily. Fourteen days later he had a recrudescence which was similarly treated. Then in another fortnight he recrudesced again when he received 4 consecutive intramuscular quinine injections followed by 25 grains a day for 5 days by mouth. In another fortnight yet another recrudescence occurred. Altogether he suffered nine attacks of the disease at 14- to 21-day intervals. He stated that he " never felt the same " after the first attack but experienced constant general weakness and headaches. On examination the liver was palpable I inch below the costal margin and red cells were reduced to 3,490,000.

Case 5.

This example shows how ever-present is the danger of serious developments in this form of malaria. Ohe month after arriving in the tropics the patient experienced his first attack. He received a few days' treatment with quinine. One month later a second attack occurred and was similarly treated. After about another month a third attack took place which began with a cold stage and rigor, constant vomiting developed with continued fever, jaundice rapidly appeared, the patient passed into a collapsed condition and on entry into hospital was found to have a greatly enlarged liver and a huge spleen.

A heavy subtertian infection was revealed by blood film and red cells were reduced to 2,000,000. Full recovery under treatment eventually occurred. He was an example of the severe type of bilious remittent fever.

Case 6.

Four months after entering the tropics the patient had his first attack of malaria. He received 20 grains quinine a day for 4 days and returned to work in 6. One month later a recurrence took place which received 5 days of treatment with quinine. Severe headaches began at this time. Following upon this attack he suffered repeated, almost daily, "low fever." This continued for 3 months, at the end of which parasites were still discoverable in the blood. He had by then become debilitated. He was unfit for work, was pale and very easily exhausted. The spleen was palpable and red cells were reduced to 3,410,000. Hb per centage was 71.

Case 7.

Some patients decline rapidly. Three weeks after entering the tropics the patient had his first attack of malaria which was treated with quinine. Three weeks later a second attack took place. Following these he developed a marked splenomegaly, a red cell count of 2,000,000 and became disabled.

Aspects of Treatment.

The accepted therapeutic principle is to treat an infection with energy as soon as possible. This is true of serotherapy and of modern chemotherapy, and, apart from the obvious logic, it needs little experience to prove its truth in subtertian malaria. For this reason we strongly urge the practice of immediate thorough treatment upon early clinical diagnosis, which is easily enough made in most cases. Even with clinically simple cases some workers have the bad habit of awaiting the purely academic satisfaction of a positive blood-film before beginning treatment.

The film is often negative during the first day or two or subtertian malaria. Delay increases the risk of serious illness and facilitates stronger foothold for the parasite in remote capillaries and reticulo-endothelial blood-spaces.

A dangerous fault is to treat a patient with quinine for a few days and then to return him quickly to duty feeling that efficiency is most helped thereby. Repeated recrudescence at short intervals, with its train of evil consequences, is inevitable in a high proportion of such cases. Barrowman in Malay found 89 per cent. of subtertian cases relapse after short quinine treatment but only 12 per cent. after prolonged. Hoops, in the same country, reported 77 per cent. relapses after one week of quinine treatment. In Brazil, in a series of 600 cases of Souza Pinto, all of whom received a total of 350 to 450 grains quinine spread over 2 to 3 weeks, there were frequent relapses. Connor, in Central America, reported 25 to 50 per cent. relapses after quinine treatment only as against 2 to 5 per cent. after atebrin and plasmoquine. In 283 cases, treated more thoroughly by Souza Pinto with atebrin and plasmoquine, there were no relapses. Nocht and Mayer (1937) remarked as follows: "We observed that sufferers did not fully recover during short quinine treatment; that, above all, they remained anaemic; that relapses very often occurred more quickly

and were of a more serious character, than after long quinine medication." CRAIG opposed the short term treatment of malaria, stating that the aim of the physician should be to eliminate infection. He maintained that there was no evidence that short treatment resulted in effective premunition or immunity and that it was dangerous in that it did not prevent the pernicious symptoms of subtertian malaria. It also favoured the development of carriers.

On this latter point NOCHT and MAYER (1937) stated that " the sooner that malaria is treated and the bouts of fever reduced, the less will be the number of gametocytes." SAUNDERS and DAWSON (1939) made the interesting observation that it was as easy to produce an initial fall in temperature in malaria with small doses of quinine as with large but that relapses were frequent, especially in subtertian malaria, after the short courses. For example, after treatment with quinine, 20 grains a day for 5 days, relapses occurred in 25 to 45 per cent. of cases.

With the enormously increased virulence of subtertian malaria when it affects troops engaged in warfare it appears that, if quinine is the only drug available for treatment, the problem will be but partially solved owing to the considerable relapse rate which follows quinine treatment of this form of malaria under any conditions but which would greatly increase under the conditions described. Of course, intensive and thorough quinine treatment would produce better results than light and inadequate administration, to which it has been necessary to refer as the commonest therapeutic mistake.

SYNTHETIC DRUGS.

The best results follow the employment of atebrin and plasmoquine in combination with quinine. One or two of the favourable reports passed upon them, in addition to those already quoted, may be given. In 1933 the Malaria Commission of the League of Nations reported " Quinine and atebrin seem to be about equally effective for the treatment of attacks of tertian and quartan but in subtertian malaria atebrin is undoubtedly superior to quinine." " Undoubtedly," writes MANSON-BAHR (1940), " its most striking effects are seen in the treatment of heavy infections of subtertian malaria."

BARROWMAN reported permanent cures in subtertian malaria four times more often with atebrin than with prolonged quinine. In 1933, in Malay, HOOPS found only 5 to 11 per cent. relapses (176 cases) as against the 77 per cent. already quoted after one week's quinine treatment.

In subtertian malaria, quinine is the most prompt of all drugs in lowering temperature and in bringing acute symptoms to an end. Atebrin exerts its effect a little more slowly but this takes a rather more prolonged form and recovery from the acute attack seems more complete, in many cases, than with quinine. The incidence of relapses, however, after atebrin treatment is quite definite, even though it may be lower than after treatment with quinine alone.

In regard to plasmoquine, it is unnecessary to refer at length to its use in eliminating the growing malarial reservoirs which large bodies of troops would otherwise carry about with them in the form of gametocyte carriers. Apart from this value we may mention MANIFOLD's opinion that the drug has a marked anti-relapse effect based upon his work in India in 1930 when he studied its effect on 3,000 troops in combination with quinine and concluded that it contained an anti-relapse factor. DUNCAN treated 168 cases with atebrin only and with 10·7 per cent. relapses whilst 116 cases treated with atebrin and plasmoquine showed only 5 per cent.

EXPERIMENTS IN TREATMENT.

Whilst it is well known that quinine and plasmoquine can be safely combined with advantage it is not always realised that the same is true of quinine and atebrin. By employing the two together in full doses simultaneously at the beginning of a malarial attack a stronger, intenser onslaught than could be made with either drug alone upon the infection in its early stage is possible. The two drugs, whilst both being parasiticidal, have different pharmacological properties and the benefit of the principle of summation of effect can be obtained from this joint use.

Attempting to formulate a thorough method of treatment with maximum effect at the beginning, we gave quinine hydrochloride 10 grains, t.d.s., together with atebrin, 0·1 gramme, t.d.s., for 7 days. In 200 cases this proved very satisfactory. No toxic sign or symptom appeared in any case.

The haematological changes were watched and are described below. They invariably took the form of steady improvement and in no case were any effects of "overtreatment" produced. The course of treatment was completed with 5 days of plasmoquine, 0·01 gramme, t.d.s., combined with quinine hydrochloride, 10 grains, t.d.s. Between the atebrin and the plasmoquine was a rest period of 48 hours during which quinine hydrochloride, 10 grains, t.d.s., was continued alone. Experience showed that in severe attacks the early dosages could, with advantage, be increased. For example, good results followed the same dose of atebrin four-hourly for a week instead of three times a day. Experience also showed most conclusively that cases treated as controls by quinine hydrochloride 10 grains, t.d.s., for 5 days were slower in resolving, suffered more blood-destruction and recrudesced much more frequently, proving thereby the greater ultimate economy of longer and more thorough treatment. We would emphasize the importance of the intensive simultaneous treatment of the attack at the onset with quinine and atebrin. Further experiment should show the actual practical value of plasmoquine, especially from the anti-relapse aspect. Probably it is best to make it a routine in hospital cases but optional in those treated in the field.

After the intensive treatment in the early stages of an attack we regard

as the next most important aspect of treatment that which we may call anti-relapse therapy. There is too great a tendency to clear up an acute attack and then to dismiss the case from further medical notice. Greater continuity of treatment is desirable. Special care should be taken to ensure that a man who has once had an attack of subtertian malaria takes a daily dose of an anti-relapse drug as long as he remains in the tropics, the dosage of this being possibly considerably larger than the regular "prophylactic" amount that may have to be taken every day in certain tropical areas, notably those of Africa. No matter how thorough the treatment of the acute attack, recrudescences will always be liable to occur, especially under conditions of strain, if the anti-relapse routine is not kept up, though, naturally, they will not be so frequent if the acute attack is thoroughly treated at the onset, nor will the patient be so pulled down by the attack and have so much blood-destruction to make up.

Among the 200 cases treated as described there was, during the next 6 months, almost complete disappearance of recrudescences. We were able to keep twenty-two of them under daily observation for 3 months. Some of the latter had a previous history of repeated relapses. After treatment with atebrin, quinine and plasmoquine as described, these cases were given for the next 3 months 15 grains anti-relapse quinine a day. This dosage, though doubtless too large for the conventional, produced no untoward sign or symptom in any case. On the contrary, their constantly watched blood pictures showed marked and continued improvement over the whole period. We maintained this substantial anti-relapse dosage partly because we suspected the fear of too much quinine to be without foundation. Daily experience deepened this suspicion. We have no doubt that the widespread fear of giving too much quinine and other drugs in malaria is a leading cause of much therapeutic failure and unnecessary illness. It is the outstanding mistake in the treatment today of the subtertian form of the disease which, as compared with the other forms, gives so little opportunity for tardiness or superficiality in treatment, and to make it under conditions of active warfare would be to court disaster in those regions where this form is heavily endemic. In these regions, daily prophylactic quinine is commonly taken, the popular dose for the last 40 years being 5 grains a day. There can be no doubt that for military purposes this amount is too small. Certainly, once a clinical attack has been experienced the dose should be raised considerably as an anti-relapse precaution; 10 grains daily should be the minimum for this purpose and, as quoted, 15 grains is better still. Even this is inferior to daily atebrin as a prophylactic and anti-relapse drug when given in adequate amounts. The standard dose of the latter in civilian life is 0·4 gramme a week but, again, we consider this too small for military purposes, especially on active service and after an acute attack has been experienced. For such purposes we advise 0·1 gramme daily (one tablet a day), prolonged trial having shown both the efficacy and the harmlessness of this. The transient

yellowness of the skin that some men may show on this can be disregarded Experience so far suggests that on the atebrin regime with this larger dose recrudescences are virtually abolished, even under strenuous conditions. On it, the overwhelming outbreaks of subtertian malaria that were so serious and uncontrollable in endemic areas in the last war would almost certainly be readily suppressed. Once instituted, such anti-relapse treatment must never be omitted during the tour of service. Otherwise a proportion of recrudescences is inevitable, no matter how thorough the treatment of the initial attack.

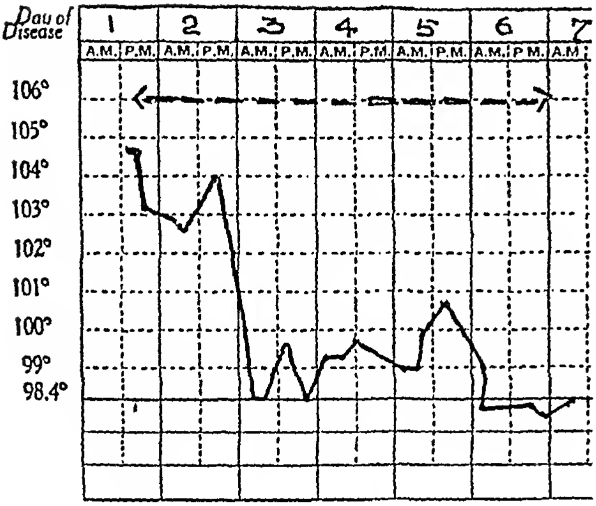
Intravenous Quinine.

In the grave syndromes, the pernicious forms of subtertian malaria, intravenous therapy is rightly regarded as essential if lives are to be saved. This is because intravenous quinine has been found the most potent means, with this drug, of destroying parasites quickly. This being so, why confine it to the pernicious forms? If we accept strong early attack on the infection as sound then, logically, intravenous therapy should be used more widely. Textbook accounts of intravenous quinine (and even those of authorities who ought to know better) are forbidding; fatal collapse, falls in blood pressure and syncope crowd each bated sentence describing the procedure. They are entirely misleading. In many cases treated as above by quinine, atebrin and plasmoquine, we substituted for the oral quinine during the first three days quinine hydrochloride, 10 grains in 10 c.c. water, intravenously each day. In pernicious conditions we often gave much more than this (for example, in one grave case, 50 grains in 4 hours). Over 300 injections were given without reaction or untoward sign or symptom in any case. They were not given particularly slowly. The scores of cases so treated were the severest ones and were definitely the quickest in clearing up. We would urge all medical men who may be concerned to be ready to use intravenous quinine in a high proportion of sharp or severe attacks of subtertian malaria in troops on active service. It would be much better to make the mistake of employing the procedure unnecessarily often under such conditions, than to allow an unreasonable timidity, based on inexperience and untruth, to deter one from carrying it out in cases which, if they had received it in adequate quantities, might have been saved from death, pernicious syndromes or prolonged ill-health. As grave cases may manifest themselves anywhere at short notice medical officers must be prepared to give intravenous quinine without delay in the field.

As Rogers (1929) has pointed out, cinchonism appears more slowly after intramuscular than after oral quinine, the former actually being the most gradual means of giving the drug, local necrosis delaying absorption. The effect of intravenous quinine, on the other hand, is not, as some have said, ephemeral. The drug is still being excreted in the urine 24 hours after an injection.

Extremely good results follow intravenous atebrin (0·3 gramme at a time)

CHARACTERISTIC TEMPERATURE CHARTS.



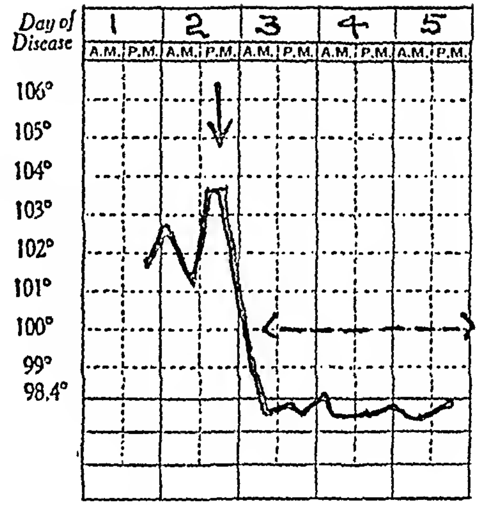
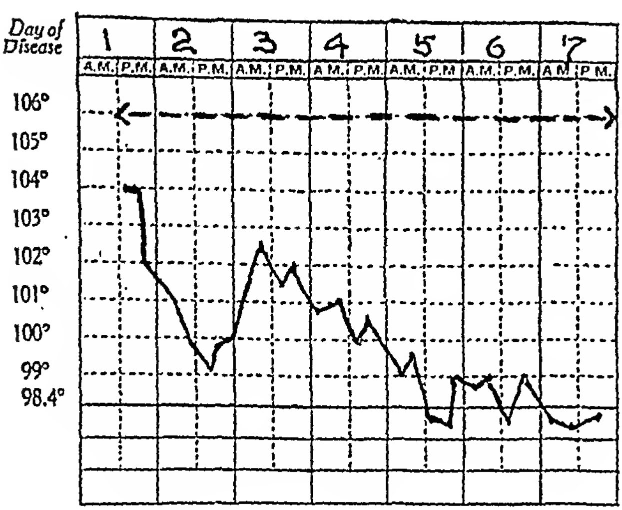


CHART 1.—Treatment with quinine hydrochloride, grains 10, t.d.s., by mouth for 6 days. One week before temperature normal.

CHART 3.—Intravenous atebrin soluble, 0·3 gramme. Arrow denotes injection. Disappearance of pyrexia and symptoms by crisis. Atebrin tabs. (0·1 gramme, t.d.s.) given by mouth for three subsequent days.



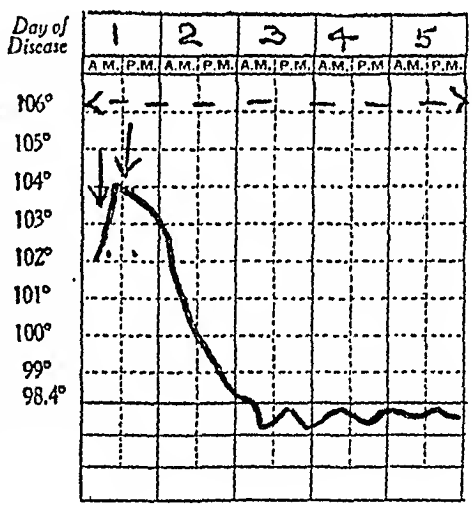


CHART 2.—Similar treatment for 7 days. Temperature normal in 1 week.

CHART 4.—Intensive combined treatment. Two intravenous injections each of 10 grains on first day (marked by arrows) quinine hydrochloride. Atebrin, 0·1 gramme and quinine hydrochloride, 10 grains given four-hourly by mouth for 5 days (dotted line). Temperature and symptoms disappear by crisis.

and ample supplies of soluble atebrin are desirable in the subtertian endemic areas.

Low Grade Forms

Low grade, recrudescent, undertreated cases improve rapidly on the methods of treatment described, quinine being omitted altogether in long standing cases to diminish the risk of blackwater fever. However, if recrudescent cases are allowed to become debilitated, though it is easy to improve them it is difficult to make them efficient soldiers again. The convalescent aspect, it should be emphasized, is an important part of the problem. Some ask, why try to eliminate recrudescences when re-infection is so likely? The answer is that less harm is done if one attack is well cleared up without recrudescence and the patient later becomes re-infected than by allowing one infection to relapse.

Grave Syndromes.

We do not wish to discuss here the treatment of the pernicious forms of subtertian malaria nor that of blackwater fever, as we are concerned in this paper with the problems of early treatment, prophylaxis and prevention.

Preventive Treatment.

The principles of active service preventive treatment have been summarized by Wenyon. They are threefold. Firstly, with rapidly moving troops drug prophylaxis alone should be relied on, it being impossible to use nets, etc, (though we would suggest that veils and gloves in bad areas are advisable; they have been used in this war by the Germans in the Ukrainian swamps). Secondly, with slowly moving troops the mosquito net can be added to drugs. together with attacks on the adult mosquito, especially with pyrethrum and paraffin sprays. Thirdly, stationary troops can add to all these the resources of anti-larval treatment.

We would particularly accent the importance of the killing of adult mosquitoes by troops under active service conditions, in contrast to anti-larval methods. S. P. James remarks that the scope of the latter is very limited in war. He advocates "direct measures applicable to the individual soldier and to the barracks, hut or tent in which he lives, such as the daily use of insecticidal sprays."

An important part of preventive work consists of the soldier's education. For him to get full benefit from the sources of help at his disposal he needs the same training in their use as he gets with his rifle or respirator. He needs a clear, simple explanation of the malaria problem and how it is combated. His instructions have to be definite, with their significance explained to him. Otherwise his morale may suffer by his vague but strong apprehension of the ill-defined but apparently inescapable perils in store for him.

Haematological Investigations.

(Report by Major J. W. Howie).

On a series of cases, treated as described above, at least two blood examinations were carried out—one before treatment and the second at the end of treatment, about 14 days later. The series consisted of 50 cases. In many of these, blood examinations were made every day during treatment. In the twenty-two cases of this series which received 15 grain anti-relapse quinine daily for 3 months after the acute attack, as described above, a blood examination was carried out at the end of this period.

On all cases Hb percentage was estimated and red and white cell and differential leucocyte counts were carried out. The fifty cases may be divided into three groups as follows.

Group 1. Acute cases—twenty-one patients.

Group 2. Recrudescent cases (length of history from first attack varying from 1 month to 1 year)—twenty-six patients.

Group 3. Low-grade cases without acute attacks—three patients.

The effects of the course of treatment may be averaged for the fifty cases. There was little variation in results from case to case.

Table I.

AVERAGED RESULTS OF TREATMENT ON FIFTY CASES (ALL TYPES).

	Before treatment.	After treatment.	Gain.
Haemoglobin	85 per cent.	91 per cent.	6 per cent.
R.B.C. ...	3,700,000	4,170,000	470,000

Daily examination showed (as is well known) that, following one paroxysm of fever, Hb percenatge may fall 10 per cent. and the red cells by 500,000. It was also clear that blood regeneration did not begin again until the attack was completely controlled. No evidence was at any time obtained that the drugs given had any harmful effect on the vascular system.

The damage done to the blood and the beneficial effect of the treatment described in long standing recrudescent cases is shown by the finding in the twenty-six patients of Group 2.

Table II.

RESULTS OF TREATMENT IN RECRUDESCENT CASES (AVERAGED).

	Before treatment.	After treatment.	Gain.
Haemoglobin	78 per cent.	88 per cent.	10 per cent.
R.B.C. ...	3,150,000	3,870,000	720,000

The blood changes of the twenty-two cases which were given 15 grains anti-relapse quinine a day for 3 months after the acute attack were as follows :

TABLE III.

AVERAGED BLOOD CHANGES IN TWENTY-TWO CASES AFTER (I) TREATMENT OF THE ACUTE ATTACK AS DESCRIBED ABOVE AND (II) SUBSEQUENT 3 MONTHS ANTI-RELAPSE QUININE, 15 GRAINS DAILY.

	Before treatment.	After 14 days.	After 3 months.
Haemoglobin	84 per cent.	92 per cent.	95 per cent.
R.B.C. ...	3,680,000	4,120,000	4,820,000

Total Gain in haemoglobin = 11 per cent. Total Gain in R.B.C.s = 1,140,000.

The low grade cases in Group 3 had in 4 months suffered a decline in red cells to below 3,500,000 in spite of 5 grains prophylactic quinine daily. They were typical of many that are seen in endemic regions. After quinine, atebrin and plasmoquine treatment as described, their counts rose to 5,360,000, 5,120,000, and 4,580,000 respectively.

Further blood examinations showed that in acute attacks leucocytosis was common whilst in chronic, recrudescent, undertreated cases, leucopaenia with relative lymphocytosis was frequent. Thorough treatment produces a restoration of the differential count to normal with a healthy polymorph-lymphocyte ratio. COOKE-PONDER'S method of Arneth counts in conjunction with reticulocyte counts suggested that in an acute attack the erythroblastic activity of the marrow is severely depressed whilst the leucoblastic function is first stimulated and then depressed and in sustained, recrudescent infection is in danger of complete exhaustion.

By daily examination during treatment on the lines described it can be seen that the red and white cell depression can be dramatically changed to normal. In a single day reticulocytes have been seen to increase from 2 per 1,000 to 50 per 1,000, whilst the increased blood sedimentation rate often noted in acute cases returned to normal.

SUMMARY.

1. The well known fact that malaria may become, in endemic areas, the most important or even decisive factor in a campaign is recalled, examples from military history are given, and that the subtertian form is the most serious in this regard is emphasized.

2. Some features of this form of malaria as it affects troops in equatorial regions are described and the considerable dangers, owing to peace-time therapeutic conventions and fears of imaginary "overtreatment," of under-treatment and consequent invalidism are stressed.

3. Illustrative cases are described together with experiments in thorough forms of treatment aiming chiefly at the quick and complete termination of an attack. It is demonstrated that quinine and atebrin can be combined in full doses without toxic effects.

4. The efficacy and harmlessness of quinine given intravenously are described and its wider use, even in the field, is advocated, as is also that of intravenous atebrin.

5. The vital importance of adequate daily drug prophylaxis throughout the stay in a subtertian area is urged, together with thorough anti-relapse treatment for all who have suffered acute attacks. The inadequacy of the popular daily 5 grains quinine is pointed out and, if quinine is going to be used for prophylaxis, the necessity for and harmlessness of 15 grains quinine a day is stated. The superiority, however, of atebrin, both for prophylaxis and anti-relapse treatment, is described and for troops on active service in subtertian areas the giving of one tablet (0·1 gramme) a day is advocated. It is suggested that, on this dosage, recrudescences would be virtually abolished and the malaria problem greatly reduced.

6. Methods of preventive treatment recently accepted by authorities are described. The desirability of educating and drilling the soldier in anti-malarial measures is emphasized.

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SULPHANILYLGUANIDINE IN THE TREATMENT OF CHOLERA.

BY

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Sulphanilylguanidine has been advocated recently in the treatment of infections of the intestinal tract. It is a water-soluble compound of the sulphonamide group which has the remarkable property of being relatively poorly absorbed from the intestinal tract (Marshall, Bratton, White and Litchfield, 1940). It is this property that leads to the obtaining of high concentrations of the drug in the intestinal tract and of low concentrations in the blood and that has resulted in it being considered particularly suited to combating intestinal disease. There have been favourable reports as to its use in the treatment of bacillary dysentery (Marshall, Bratton, Edwards and Walker, 1941, and Lyon, 1941); and Firor and Jonas (1941) have recommended it as a pre-operative preparation for surgery on the bowel. For these reasons, it was decided to give the drug a trial during an epidemic of cholera recently experienced.*

The diagnosis of cholera, in these cases, rested upon the presence of emesis, rice water stools and marked dehydration and collapse, of acute onset and occurring during the course of a known epidemic of cholera. In addition, the vibrio was demonstrated in the stools of a large number of the cases. The dosage of sulphanilylguanidine used was in accordance with that given by Lyon (1941) in bacillary dysentery. The initial dose was 0·1 gramme per kg. of estimated body weight and the maintenance dose was 0·05 gramme per kg. every 4 hours until the stools became normal. The stools were considered to be normal when they had become faecal in character, semi-solid or solid

* The sulphanilylguanidine used in this study was kindly supplied by E. R. Squibb & Sons through their agents, H. J. Foster & Co., Bombay.

TABLE III.

MORBIDITY.

	Control Group.						Sulphanilylguanidine-treated Group.					
Age group in years ...	0–10	11–20	21–30	31–40	* 41–50	50 over	0–10	11–20	21–30	31–40	* 41–50	* 50 over
Average age	5·7	17·6	28·3	37·5	50·0	—	6	14·7	25·1	38·3	45·0	60·0
Number hours ill before admission	23·5	17·0	12·3	62·5	14·0	—	12	12·8	12·4	9·0	2·0	9·0
Number hours in hospital	66·2	104·0	91·8	56·0	46·0	—	78·7	110·6	132·9	120·0	9·0	17·6
Temperature over 101°F.	27·2%	50·0%	50·0%	0·0%	0·0%	—	34·7%	32·5%	40·0%	66·6%	0·0%	100·0%
Urinary suppression more than 12 hours	36·3%	33·3%	66·6%	100·0%	0·0%	—	21·7%	16·6%	80·0%	66·6%	0·0%	100·0%
Number hours before stool became normal†	49·7	62·4	80·0	36·0	36·0	—	48·7	58·0	84·0	32·0	—	108·0
Intravenous saline given	36·3%	83·3%	100·0%	100·0%	100·0%	—	60·8%	100·0%	100·0%	100·0%	100·0%	100·0%
Subcutaneous saline given	81·8%	0·0%	0·0%	0·0%	0·0%	—	73·9%	8·3%	0·0%	0·0%	0·0%	0·0%
Average blood specific gravity‡	1058	1061·6	1062·3	1064·0	1062·0	—	1060·0	1061·8	1062·3	1056·8	1066·0	1063·0
Amount sulphaguanidine given in grammes	—	—	—	—	—	—	13·9	30·8	46·8	36·0	8·0	83·0

Notes.—* One case only in these groups. † Two semi-solid stools were considered as normal.
‡ Blood specific gravity was taken immediately on admission.

in the number of cases in the control and in the sulphanilylguanidine-treated groups under each age heading, causing some difficulty in interpreting the results. However, in general the groups are fairly comparable and no significant differences can be seen either as to the types and severity of the cases treated with or without sulphanilylguanidine in the various age groups nor in the end results obtained.

In consequence, it may be concluded that sulphanilylguanidine has no demonstrated value in the treatment of cholera. Such differences as are seen are considered not to be sufficient to be of statistical significance.

SUMMARY.

1. In an epidemic of cholera, fifty cases were treated with sulphanilylguanidine and eighty-eight cases served as controls.

2. The treatment of cholera by sulphanilylguanidine was not shown to be of value.

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ADDITIONAL NOTE.—Since completing this investigation and preparing this communication, an article entitled "Sulphanilylguanidine in cholera," by CHOPRA, DE MONTE, GUPTA and CHATTERJI has appeared (*Ind. med. Gazette*, **76**, 712-713, Dec., 1941). In this article the authors claim a definite reduction in the mortality of cholera when treated by sulphanilylguanidine, but as their cases were not divided into age groups an exact comparison with the results recorded in this paper is not possible.

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ANTI-TYPHUS VACCINATION OF GUINEAPIGS WITH VACCINE PREPARED FROM INFECTED GERBILS.

BY

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In a previous communication† experiments were described showing that the common gerbils of South Africa—*Tatera brantsi* and *T. afra*—are susceptible to epidemic typhus, to murine typhus and to tick-bite fever. In these experiments it was found that after intraperitoneal inoculation very profuse growths of rickettsiae often occurred. By exposing the animals to X-rays before inoculation it was found that very prolific growths of rickettsiae occurred in almost all the animals inoculated. As large numbers are readily available it was considered that the gerbil would be a suitable animal to use for the preparation of prophylactic vaccines against epidemic typhus, and against tick-bite fever, in the same way that white rats were used in the preparation of anti-typhus vaccine by the Zinsser-Castaneda method. This paper describes experiments to test the efficacy of a vaccine so prepared in protecting guineapigs against epidemic typhus.

* The authors are grateful to Sir EDWARD THORNTON, Director General of Medical Services, and to Dr. E. H. CLUVER, Director of the South African Institute for Medical Research, for their interest and permission to publish this paper.

† GEAR, J. & DAVIS, D. H. S. (1942). The susceptibility of the South African gerbils (genus *Tatera*) to rickettsial diseases and their use in the preparation of anti-typhus vaccine. *Trans. R. soc. trop. Med. Hyg.*, 36, 1.

Method of Preparation of Vaccine.

The gerbils used in these experiments were caught wild and were kept for periods varying from one day to two weeks before being used, except that young ones caught were kept until they were adult.

Before inoculation the gerbils were exposed to X-rays for 15 minutes, the total dose given being 600 R.

They were then inoculated intraperitoneally with 1 c.c. of a suspension of infective brain. This was prepared by emulsifying the brain of a guineapig killed on the third day of the fever of an attack of epidemic typhus, in 15 c.c. of normal saline. The gerbils were then kept in an isolation room whose temperature ranged from 70° to 80° F. From the 5th to the 10th day the majority of them became sick and within a day or two most were moribund, when they were killed by chloroform. The peritoneal cavity was opened and scraped with a scalpel having a blunt slightly roughened cutting edge, and then washed out with 0·2 per cent. formol saline. From 20 to 40 c.c. of a suspension of rickettsiae and cells were obtained from each gerbil.

This suspension was then centrifuged in an angle centrifuge at 3,000 to 4,000 revolutions per minute for 1 hour. This threw down most of the rickettsiae and other suspended particles. The supernatant fluid was pipetted off and the deposit resuspended in 10 c.c. of 0·2 per cent. formol saline and then centrifuged at 1,000 revolutions per minute for 20 to 30 minutes. The supernatant fluid was pipetted off and conserved. The deposit was resuspended and again centrifuged slowly as before. The supernatant fluid was pipetted off and added to the previous supernatant fluid. The deposit, consisting mostly of epithelial and inflammatory cells, was now discarded.

The rickettsiae suspension was then centrifuged rapidly 3,000 to 4,000 revolutions for 1 hour to deposit the rickettsiae. The supernatant fluid was discarded and 0·1 per cent. formol saline added to the deposit, consisting now of rickettsiae, until the suspension had an opacity approximately equal to 1,000 million *B. coli* per c.c. This suspension of rickettsiae was used as the vaccine for the following experiment.

Experiment 1.

Six young adult male guineapigs were inoculated with 1 c.c. of the vaccine at intervals of 1 week, until each had received a total of three doses. They were rested for 1 month during which one died of an intercurrent infection. Then the test dose was inoculated intraperitoneally. This challenge dose was prepared from the brain of a pig suffering from epidemic typhus, removed on the 3rd day of fever and emulsified and suspended in 10 c.c. of normal saline. Tenfold and hundredfold dilutions of this suspension were then prepared.

The five test guineapigs were each inoculated with 1 c.c. of the original suspension.

Five control guineapigs were inoculated with the same dose and five with the tenfold dilution and five with 1 c.c. of the hundredfold dilution.

None of the vaccinated guineapigs reacted. One of the control guineapigs died of an intercurrent infection and, of the fourteen remaining, twelve reacted.

The two control animals that failed to react were inoculated with the hundredfold dilution of the test dose. It is concluded therefore that each of the vaccinated animals was inoculated with over 10 and approximately 100 minimum infective doses. Against this dose the vaccination was effective in conferring protection.

It can therefore be concluded that an effective anti-typhus vaccine can be prepared from the peritoneal washings of gerbils infected after exposure to X-rays by intraperitoneal inoculation.

TABLE.

Dilution.	Vaccinated guineapigs.		Unvaccinated control guineapigs.	
	Number susceptible.		Number susceptible.	
Undiluted infective suspension	5	0	5	5
Diluted 1/10	–	–	(5) 4	4
Diluted 1/100	–	–	5	3
		Total	14	12

As it was considered that the yield of rickettsial suspension could be greatly increased by *intrapleural inoculation* as well as by the intraperitoneal route, several gerbils were inoculated intrapleurally on one side (the right) as well as intraperitoneally. A profuse growth of rickettsiae occurred in the pleura and the pleural fluid was very rich in rickettsiae. However, the additional yield was relatively so small, principally because the pleural cavity of the gerbil in relation to the peritoneal cavity is so small, that it was not considered worth the additional procedures involved to adopt this as a routine measure.

DISCUSSION.

It will be recalled that the Zinsser-Castaneda method of preparing vaccines by the use of white rats could not be applied to the making of vaccines from strains of epidemic typhus, because even after exposure to X-rays the growth of rickettsiae in the peritoneal cavity was not sufficiently profuse for this purpose. Nor was any other laboratory animal known that could be used. These experiments have indicated that an effective vaccine against epidemic typhus can be prepared from the common South African gerbil.

In addition the findings suggest that the gerbil may prove to be a very suitable experimental animal for investigation of the typhus group of fevers as it appears to be much more susceptible than the guineapig, the animal most commonly used for this work.

It is estimated that there are many millions of these rodents in South Africa. As already noted, the aid of several public health authorities in South Africa has been enlisted and several hundred gerbils have been sent to the South African Institute for Medical Research for the preparation of anti-typhus prophylactic vaccine, which is now being produced on a large scale.

Nine persons, all laboratory workers handling virulent typhus strains, have been inoculated with this vaccine. Each received four doses of 1 c.c. at weekly intervals. In all of them there were slight local reactions at the site of the inoculation—in the deltoid region—but this was less severe than that experienced after vaccination with T.A.B. endotoxoid vaccine, except in one instance. In this man, who was an allergic subject, the local reaction after the second injection was severe, involving the outer aspect of the arm from the shoulder to the elbow. His subsequent injections were therefore given intradermally and were followed by much less intense local reactions. There was no general disturbance associated with the vaccination in any of the vaccinated persons, except in one man who complained of slight headache on the day following the second injection, but this was not sufficiently severe to cause him to cease work. The serological response of these persons and several others will be reported in a later communication.

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SUB-CLINICAL ANAEMIA OF SCHOOL-CHILDREN IN SOUTHERN RHODESIA.

BY

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For various reasons the haematological state of school-children in Southern Rhodesia might be expected to differ from that in other parts of the world. The greater part of the area inhabited by European and Coloured races—" Coloured " here means mixed European and Bantu descent, but also includes Asiatic—is a plateau varying from about 3,500 to 5,000 feet above sea level and situate a few degrees North of the tropic of Capricorn. The climate in this area, in spite of its tropical latitude, is tempered by altitude, so that the mean annual temperature varies between 63° and 68° F. and there are definite wet and dry seasons. The rains fall during the warm season and the alternating periods of hot sunshine and heavy downpours of rain acting on land, large areas of which are relatively devoid of heavy vegetation, favour rapid erosion of the surface soil, which is therefore in many parts deficient in soluble salts of minerals such as calcium, phosphorus and iodine. Another effect of the rains falling during the warm season is the promotion of ideal conditions for mosquitoes and snails to propagate at this time and transmit the endemic

* The writer wishes to express his thanks to Dr. R. M. Morris, Acting Medical Director of the Southern Rhodesian Medical Service, for permission to submit this paper for publication.

parasitic diseases of malaria (and blackwater fever) and schistosomiasis, and for the development of ankylostome ova in the soil.

Throughout this area education of European and Coloured children of school age is compulsory and free facilities in this respect are provided by the Government for the whole population. There are also several private schools, most of which are conducted by religious denominations; many of these schools are subsidised by the Government and they are all liable to Government inspection and control in accordance with local legislation. Owing to the scattered nature of the population about 32 per cent. of all school-children are accommodated during term-time in boarding hostels attached to the larger schools. Accommodation is also provided in these boarding establishments for children domiciled in other territories on the Northern and Eastern boundaries of the Colony, but these children form a very small proportion of the school population.

In any haematological survey of this school population one must therefore take into account the relative effects of race, age, climate, altitude, diet and parasitic infection. BLACKIE (1936) considers that the salient anaemia-producing influences in Southern Rhodesia are (1) Disease. (2) Climate. (3) Diet. (4) Altitude; and that a surprising number of individuals suffer from a subnormal state of health attributable to anaemia.

A preliminary survey of school-children in Southern Rhodesia was made by the writer in 1938 using Tallqvist's method, in conjunction with clinical examination and laboratory examination of the blood-smear, urine and stool. From this survey it was surmised that a condition of sub-clinical anaemia was the rule rather than the exception, but no definite conclusions were drawn owing to the fact that a large degree of human error is possible with Tallqvist's method, so it was decided to do a further survey using a more reliable method. The ideal would have been a full haematological examination, but this was impossible in conjunction with routine medical inspection of school-children over an area which is approximately twice that of Great Britain and in which an average of 2½ miles is travelled for every child examined at routine inspection.

Technique Adopted.—Newcomer's method was chosen on account of the ease with which this technique can be employed in conjunction with routine clinical examination of large numbers of children. There are many possibilities of error in this method, but if technique is methodical, the glass standard kept in the dark when not in use, and the instrument always maintained in perfect adjustment, the only unavoidable error is subjective variation on the part of the persons estimating the colour standard of the solution. In this survey, all estimations were made by the same person. Haemoglobin indices were recorded in grammes of haemoglobin per 100 c.c. of whole blood in order to avoid the confusion which results from recording the index as a percentage of the "normal" which is 13·8 grammes per 100 c.c. of blood on Haldane's scale

and 15·3 grammes on the Sahli-Leitz scale. A blood-smear taken at the same time was examined for malaria parasites and the eosinophil percentage recorded. Routine examination of the urine and stool for helminthic ova was carried out until the middle of the year, when the extra laboratory work incurred by the examination of recruits for the military forces precluded any further routine laboratory examination of school-children.

Standards Determined by Other Observers.—DAVIDSON, FULLERTON and CAMPBELL (1935), working in the poorer districts in Scotland, took 95 per cent. on the Haldane scale (13·1 grammes per cent.) as a normal standard for school-children, and the range of normal variation as 10 per cent., *i.e.*, from 11·73 grammes per cent. to 14·49 grammes per cent. GILCHRIST (1939) considers that 90 per cent. on the Sahli scale (13·77 grammes per cent.) should be taken as the lower limit of normal haemoglobin level, since the Hb of Leyton (England) school-children under this level was raised from 10 per cent. to 15 per cent. by the administration of iron and ammonium citrate.

ROBERTS, STONE & BOWLER (1938), working in Bristol (England) among school-children and including as many as possible of the worst nourished children, found only 9 out of 189 (4·8 per cent.) children who showed lower haemoglobin indices than 80 per cent. on the Haldane Scale (11·04 grammes per cent.).

These standards were all estimated at or near sea level in the temperate zone. It is well known that altitude tends to increase the red cell count and haemoglobin index. Thus LIKNAITZKY (1934) working at an altitude of 5,750 feet (Johannesburg) found that in a group of sixty healthy males between 17 and 30 years of age, the mean value for red cells was 5,990,000 per c.mm.; other things being equal, this should co-exist with a haemoglobin index from 10 to 20 per cent. over that found at sea level. In a small group of healthy male adults between 18 and 25 years of age in Salisbury (Southern Rhodesia) (5,000 feet) BLACKIE (1936) found the average red cell count 5,300,000 per c.mm., while for practical purposes the corresponding figure for females was 5,000,000. Thus, he says, in assessing anaemic states in Southern Rhodesia, no special allowance need be made for altitude. Incidentally, these figures are very similar to OSGOOD's (1935) average figures, taken from apparently healthy persons, native born and living in or near Portland, Oregon, when the figure for 259 males from 14 to 30 years was estimated at 5,420,000, and that for 152 females of the same age was 4,830,000. OSGOOD's average figure for haemoglobin index for 215 boys and girls from 4 to 13 years was 11·92 grammes per cent. with a range of values from 10 to 14 grammes. The range from 14 to 30 years varied from 14 to 18 grammes for males and 11·5 to 16 grammes for females.

The large range of haemoglobin values in normal adults over a period of time has been investigated by INGERSOLL (1936), who determined the haemoglobin indices of thirty young adult medical students regarded as being in

excellent health and determinations were made weekly or more often from October to January. The maximum and minimum haemoglobin ranges varied between 0·4 and 2·7 grammes per 100 c.c. Fourteen had a range up to 1 gramme and sixteen above this amount. The main outcome of these studies is to show the absurdity of relying on single readings to decide a clinical condition by means of blood examination. (The differences were, however, greater in females than in males and this fact seems to show that the minimum values probably indicated sub-clinical anaemia in the same way that an individual's weight may vary from week to week in the absence of any definite signs of ill-health detectable by ordinary clinical methods; in other words, it shows depletion of reserve stamina).

NEAVE KINGSBURY (1939), in a survey of the haemoglobin indices of about 3,000 Tamil and 1,200 Malay school-children in the Malay States, using Tallqvist's method, found the average percentage of Tamil school-children on four groups of estates were 68·6, 68·7, 69·7 and 68·4, while that of the Malay school-children was 76·7. These figures are above those determined by the writer for 941 European school-children in Southern Rhodesia by Tallqvist's method in 1938, the average for which was 67·7 per cent. with a standard deviation of 8·7, while 302 of these children (32·1 per cent.) had indices of 60 per cent. or less, although the Malay States would *prima facie* appear to offer greater anaemia-producing influences than Southern Rhodesia. NEAVE KINGSBURY concludes that the roles of malaria and helminthiasis (gauged by palpable spleen or eosinophilia, but not by examination of stools) in the causation of anaemia on the estates may have been over-stressed since those children with non-palpable spleens, and an eosinophil count of 5 per cent. or less, showed an average haemoglobin index of 69 per cent. while the whole group (which included all the parasite-infected) had an average of 68·7 per cent.—only 0·3 per cent. lower. Much of the widespread anaemia among young Tamils he believed to be referable to sub-nutrition, while the low spleen rates among these children indicated in some degree the efficacy of malarial control on estates, since the spleen rate in the Malay schools was about four to twenty times higher than in the Tamil schools where the haemoglobin indices were from 8 to 9 per cent. lower.

Standards Adopted in the Present Investigation.—In the absence of a definite normal standard for healthy persons living under ideal conditions in Southern Rhodesia it was decided to adopt the average figure for all the 2,173 European school-children examined in this survey (13·49 grammes per cent.—equivalent to 97·7 per cent. on the Haldane scale) as the normal standard, and, in accordance with the procedure of DAVIDSON and his co-workers (1935), allowance was made for a range of normality of 10 per cent. on the Haldane scale (1·38 grammes Hb per cent.) above and below. The lower limit of normality would therefore be 12·11 grammes Hb per 100 c.c.—equivalent to 87·7 per cent. Haldane or 79·1 per cent. Sahli-Leitz, but since the haemoglobin

indices in the accompanying tables are listed in groups varying to the extent of 0·5 gramme Hb, *the normal range adopted has been from* 12 *to* 14·9 *grammes Hb per* 100 *c.c. of blood, any figure below* 12 *being considered as anaemia.*

Results Obtained.—Table I shows that the average Hb index of all the 2,173 European school-children examined was 13·49 grammes per cent. with a standard deviation of 1·55. 320 (14·7 per cent.) of these children were

TABLE I.

EUROPEAN GROUP (INCLUDES ALL EUROPEAN SUB-GROUPS).

Haemoglobin (grammes per 100 c.c.).	Numbers of Children in Age-groups.				Total (all ages).
	Under 10 years.	Over 10 years and under 13 years.	Over 13 years and under 15 years.	Over 15 years.	
7·0– 7·4	1	—	—	—	1
7·5– 7·9	—	—	—	—	—
8·0– 8·4	—	—	—	—	—
8·5– 8·9	1	—	—	—	1
9·0– 9·4	1	1	2	—	4
9·5– 9·9	3	4	3	—	10
10·0–10·4	13	7	5	—	25
10·5–10·9	29	33	13	2	77
11·0–11·4	29	23	15	3	70
11·5–11·9	66	34	25	7	132
12·0–12·4	65	64	44	6	179
12·5–12·9	136	86	74	25	321
13·0–13·4	114	77	62	35	288
13·5–13·9	127	75	80	35	317
14·0–14·4	48	36	36	17	137
14·5–14·9	82	65	41	52	240
15·0–15·4	39	50	52	36	177
15·5–15·9	10	13	22	25	70
16·0–16·4	8	13	15	25	61
16·5–16·9	9	5	5	11	30
17·0–17·4	—	4	8	14	26
17·5–17·9	—	—	1	1	2
18·0–18·4	—	—	—	2	2
18·5–18·9	—	1	—	1	2
19·0–19·4	—	1	—	—	1
Total	781	592	503	297	2,173
Mean Hb index ...	13·16	13·30	13·56	14·54	13·49
Standard deviation...	1·36	1·53	1·55	1·48	1·55
Percentage with parasitic infection ...	8·19	13·52	12·52	14·47	11·50

anaemic, while the extreme range from 7·2 to 19·2 grammes seems to show that the standard adopted for anaemia is probably on the low side.

Table II gives haemoglobin estimations of all the 304 Coloured school-children examined. The average index of 12·99 with a standard deviation of 1·44 is somewhat lower than that of the Europeans, while the number of 70

(23 per cent.) showing anaemia is proportionately higher, and the extreme range from 6·7 to 17·2 grammes per cent. again points to the standard for anaemia being a low one.

The figures for both European and Coloured groups appear to rise gradually with increasing age.

TABLE II.

COLOURED GROUP (INCLUDES ALL COLOURED SUB-GROUPS)

Haemoglobin (grammes per 100 c.c.).	Numbers of Children in Age-groups.				Total (all ages).
	Under 10 years.	Over 10 years and under 13 years.	Over 13 years and under 15 years.	Over 15 years.	
6·5– 6·9	1	—	—	—	1
7·0– 7·4	—	—	—	—	—
7·5– 7·9	—	—	—	—	—
8·0– 8·4	—	—	—	—	—
8·5– 8·9	1	—	—	—	1
9·0– 9·4	—	—	—	—	—
9·5– 9·9	—	—	—	1	1
10·0–10·4	3	—	—	—	3
10·5–10·9	7	3	2	—	12
11·0–11·4	13	3	3	—	19
11·5–11·9	20	7	5	1	33
12·0–12·4	13	13	6	2	34
12·5–12·9	28	14	4	9	55
13·0–13·4	15	13	11	1	40
13·5–13·9	21	4	12	7	44
14·0–14·4	5	6	3	2	16
14·5–14·9	3	7	4	1	15
15·0–15·4	7	1	3	4	15
15·5–15·9	—	1	1	2	4
16·0–16·4	2	1	—	1	4
16·5–16·9	2	—	—	1	3
17·0–17·4	—	—	2	2	4
Total	141	73	56	34	304
Mean Hb index ...	12·68	12·95	13·29	13·88	12·99
Standard deviation...	1·46	1·20	1·39	1·64	1·44
Percentage with parasitic infection ...	13·48	12·33	12·50	17·65	13·49

11·5 per cent. of the European children and 13·5 per cent. of the Coloured children were proved to have parasitic infection (*i.e.*, helminthic ova—mainly schistosome—in urine or stool, or the presence of parasites in the blood smear, or a palpable spleen). The Hb indices of these children with proved parasitic infection are shown in Table III (Europeans) and Table IV (Coloured). The average Hb index of the 250 Europeans in Table III is 13·2 with a standard deviation of 1·59, while 58 (23·2 per cent.) of these children were anaemic

TABLE III.

SUB-GROUP OF EUROPEANS PROVED TO HAVE PARASITIC INFECTION

Haemoglobin (grammes per 100 c.c.).	Number of Children in Age-Groups.				Total (all ages).
	Under 10 years.	Over 10 years and under 13 years.	Over 13 years and under 15 years.	Over 15 years.	
7·0– 7·4	1	—	—	—	1
7·5– 7·9	—	—	—	—	—
8·0– 8·4	—	—	—	—	—
8·5– 8·9	—	—	—	—	—
9·0– 9·4	—	1	—	—	1
9·5– 9·9	—	—	1	—	1
10·0–10·4	2	—	2	—	4
10·5–10·9	5	3	2	—	10
11·0–11·4	9	5	5	1	20
11·5–11·9	11	7	3	—	21
12·0–12·4	6	19	5	3	33
12·5–12·9	5	7	10	3	25
13·0–13·4	6	14	11	10	41
13·5–13·9	5	9	4	2	20
14·0–14·4	4	2	4	1	11
14·5–14·9	5	5	5	8	23
15·0–15·4	5	4	5	10	24
15·5–15·9	—	3	2	1	6
16·0–16·4	—	1	2	3	6
16·5–16·9	—	—	—	1	1
17·0–17·4	—	—	1	—	1
17·5–17·9	—	—	1	—	1
Total	64	80	63	43	250
Mean Hb index ...	12·52	12·93	13·18	14·19	13·20
Standard deviation...	1·54	1·37	1·66	1·28	1·59

TABLE IV.

SUB-GROUP OF COLOURED PROVED TO HAVE PARASITIC INFECTION.

Haemoglobin (grammes per 100 c.c.).	Numbers of Children in Age-groups.				Total (all ages).
	Under 10 years.	Over 10 years and under 13 years.	Over 13 years and under 15 years.	Over 15 years.	
6·5– 6·9	1	—	—	—	1
7·0– 7·4	—	—	—	—	—
7·5– 7·9	—	—	—	—	—
8·0– 8·4	—	—	—	—	—
8·5– 8·9	1	—	—	—	1
9·0– 9·4	—	—	—	—	—
9·5– 9·9	—	—	—	1	1
10·0–10·4	1	—	—	—	1
10·5–10·9	2	1	1	—	4
11·0–11·4	2	—	2	—	4
11·5–11·9	2	1	—	—	3
12·0–12·4	—	2	2	1	5
12·5–12·9	4	2	—	1	7
13·0–13·4	3	2	1	—	6
13·5–13·9	1	—	—	—	1
14·0–14·4	—	1	1	—	2
14·5–14·9	—	—	—	—	—
15·0–15·4	2	—	—	3	5
Total	19	9	7	6	41
Mean Hb index ...	11·96	12·53	12·13	13·37	12·08
Standard deviation...	2·02	0·94	1·15	2·06	1·78

and the range varied from 7·2 to 17·7 grammes per cent. The average index for the forty-one Coloured children in Table IV is 12·08 with a standard deviation of 1·78; 15 (36·6 per cent.) of these were anaemic and the extreme range varied from 6·7 to 15·2 grammes per cent.

Table V gives the Hb estimations of 1,276 European children, selected from the group of 2,173 children in Table I on account of the fact that they were examined during the warm season (*i.e.*, between 1st October and 30th

TABLE V.

SUB-GROUP OF EUROPEANS EXAMINED DURING SUMMER.

Haemoglobin (grammes per 100 c.c.).	Numbers of Children in Age-groups.				Total (all ages).
	Under 10 years.	Over 10 years and under 13 years.	Over 13 years and under 15 years.	Over 15 years.	
9·0– 9·4	—	—	2	—	2
9·5– 9·9	—	4	2	—	6
10·0–10·4	4	6	3	—	13
10·5–10·9	4	27	10	2	43
11·0–11·4	8	21	12	3	44
11·5–11·9	32	28	26	6	22
12·0–12·4	31	67	39	7	144
12·5–12·9	59	46	62	16	183
13·0–13·4	51	74	51	29	205
13·5–13·9	43	46	42	23	154
14·0–14·4	25	26	26	10	87
14·5–14·9	42	49	29	38	158
15·0–15·4	10	11	18	24	63
15·5–15·9	2	8	13	14	37
16·0–16·4	3	8	7	12	30
16·5–16·9	1	3	1	4	9
17·0–17·4	—	1	1	2	4
17·5–17·9	—	—	1	—	1
18·0–18·4	—	—	—	1	1
Total	315	425	345	191	1,276
Mean Hb index ...	13·20	13·03	13·23	14·19	13·38
Standard deviation...	1·17	1·43	1·40	1·36	1·36
Percentage with parasitic infection ...	8·25	13·42	13·04	16·75	12·54

April). This sub-grouping was done in order to throw light on BLACKIE'S suggestion that anaemia among Europeans in Southern Rhodesia may be due to climatic factors which, according to his theory, lower the blood chlorides through excessive perspiration and thus render the gastric mucosa deficient in hydrochloric acid. The average index of this sub-group was 13·38 grammes per cent. with a standard deviation of 1·36, with an extreme range from 9·2 to 18·2 grammes per cent.; 130 (10·2 per cent.) were anaemic and the percentage

infected with parasites was 12·54—somewhat higher than in the whole group. These figures, even without taking into consideration the greater proportion of children with parasitic infection, do not lend any support to BLACKIE'S theory because the proportion of children showing anaemia is less than that in the whole group of European children examined.

From the two main groups of (1) Europeans in Table I, and (2) Coloured in Table II, 924 (Europeans) and 90 (Coloured) children have been selected

TABLE VI.

SUB-GROUP OF EUROPEAN BOARDERS.

Haemoglobin (grammes per 100 c.c.).	Numbers of Children in Age-Groups.				Total (all ages).
	Under 10 years.	Over 10 years and under 13 years.	Over 13 years and under 15 years.	Over 15 years.	
9·0– 9·4	—	—	2	—	2
9·5– 9·9	—	—	1	—	1
10·0–10·4	4	1	3	—	8
10·5–10·9	4	10	6	1	21
11·0–11·4	9	8	4	3	24
11·5–11·9	34	9	14	4	61
12·0–12·4	21	43	33	3	100
12·5–12·9	43	33	39	17	132
13·0–13·4	44	36	29	25	134
13·5–13·9	21	39	42	24	126
14·0–14·4	10	33	12	11	66
14·5–14·9	18	32	19	33	102
15·0–15·4	7	13	19	29	68
15·5–15·9	1	4	9	9	23
16·0–16·4	—	6	4	21	31
16·5–16·9	—	1	3	8	12
17·0–17·4	—	—	3	8	11
17·5–17·9	—	—	—	1	1
18·0–18·4	—	—	—	1	1
Total	216	268	242	198	924
Mean Hb index ...	13·52	13·35	13·36	14·51	13·49
Standard deviation...	—	—	—	—	1·47
Percentage with parasitic infection ...	15·3	16·4	14·5	14·6	15·3

respectively on account of the fact that they were resident in school boarding hostels at the time of the investigation; these sub-groups are shown in Table VI (European boarders) and Table VIII (Coloured boarders), while Table VII shows a further sub-group of those European boarders who were proved to have parasitic infection. The dietaries of these children resident in boarding hostels were surveyed during the year under consideration so these children therefore act to some extent as a control group in so far as their dietaries are concerned.

TABLE VII.

SUB-GROUP OF EUROPEAN BOARDERS PROVED TO HAVE PARASITIC INFECTION.

Haemoglobin (grammes per 100 c.c.).	Numbers of Children in Age-groups.				Total (all ages).
	Under 10 years.	Over 10 years and under 13 years.	Over 13 years and under 15 years.	Over 15 years.	
9·5– 9·9	—	—	1	—	1
10·0–10·4	2	—	—	—	2
10·5–10·9	—	—	—	—	—
11·0–11·4	2	1	1	1	5
11·5–11·9	5	2	2	—	9
12·0–12·4	3	11	3	1	18
12·5–12·9	5	4	7	3	19
13·0–13·4	4	9	6	5	24
13·5–13·9	5	4	4	5	18
14·0–14·4	1	3	3	1	8
14·5–14·9	3	4	2	5	14
15·0–15·4	3	4	2	6	15
15·5–15·9	—	1	2	—	3
16·0–16·4	—	1	1	1	3
16·5–16·9	—	—	—	—	—
17·0–17·4	—	—	1	—	1
17·5–17·9	—	—	—	—	—
18·0–18·4	—	—	—	1	1
Total	33	44	35	29	141
Mean Hb index ...	13·41	13·35	13·46	14·11	13·43
Standard deviation...	—	—	—	—	1·42

TABLE VIII.

SUB-GROUP OF COLOURED BOARDERS.

Haemoglobin (grammes per 100 c.c.).	Numbers of Children in Age-groups.				Total (all ages).
	Under 10 years.	Over 10 years and under 13 years.	Over 13 years and under 15 years.	Over 15 years.	
10·5–10·9	2	1	—	—	3
11·0–11·4	4	—	1	—	5
11·5–11·9	8	5	2	1	16
12·0–12·4	5	6	1	1	13
12·5–12·9	5	1	3	5	14
13·0–13·4	5	8	4	—	17
13·5–13·9	4	1	2	6	13
14·0–14·4	1	—	1	—	2
14·5–14·9	—	—	2	—	2
15·0–15·4	—	1	1	1	3
15·5–15·9	—	—	—	—	—
16·0–16·4	—	—	—	—	—
16·5–16·9	1	—	—	—	1
17·0–17·4	—	—	—	1	1
Total	35	23	17	15	90
Mean Hb Index ...	12·46	12·59	13·17	13·47	12·79
Standard deviation...	2·39	2·00	2·18	2·58	1·19
Percentage with parasitic infection ...	14·3	4·4	5·9	6·7	8·9

The average index for the 924 European boarders listed in Table VI is 13·49 (precisely the same as that of the whole group in Table I) with a standard deviation of 1·46; 117 (12·7 per cent.) of these were anaemic, while 15·3 per cent. were infected with parasites. The proportion of anaemic children was therefore less than that of the whole group in Table I (14·7 per cent.) while at the same time the proportion of parasite-infected children was greater (cf. 11·5 per cent. in Table I).

The average index for the sub-group of 141 parasite-infected European boarders listed on Table VII is 13·43 with a standard deviation of 1·42, while the number of anaemic children was 17 (12·1 per cent.). Parasitic infection among European boarders therefore does not appear to have any marked effect on the haemoglobin concentration of the blood.

The average index of the 90 Coloured boarders, listed on Table VIII, is 12·79 with a standard deviation of 1·19; 24 (26·7 per cent.) of these children were anaemic (as compared with 23 per cent. in the whole group in Table II) while the parasite-infected proportion was 8·9 per cent. (as compared with 13·49 per cent. in Table II). These figures do not demonstrate the higher haemoglobin indices which might be expected among Coloured boarders as compared with the whole group of Coloured children in Table II, and this matter will be discussed below.

Discussion.

Age.

The ages of the children examined varied from about 5 to 17 years. For convenience, the children were divided into four age-groups :—

(1) Under 10 years—average approximately 7½ years.
(2) Over 10 years and under 13 years, average approximately 11½ years.
(3) Over 13 years and under 15 years, average approximately 14 years.
(4) Over 15 years, average approximately 16 years.

Of the Europeans, girls numbered 539 (24·8 per cent.) and of the Coloured children 105 (34·5 per cent.). The girls examined were mostly below the age of puberty, and since no difference in haemoglobin concentration between the sexes may be expected at this age, the figures for boys and girls are shown together.

The effect of age between 6 years and 18 years was shown by the figures of Davidson and his co-workers (1935) to raise the average haemoglobin index from 87·4 per cent. (Haldane) at 6 years to 93 per cent. at 11 years, and thence, in males of 12 to 18 years, to 98·8 per cent., while females of this age show a decrease to 91·7 per cent. This finding seems to be the general rule among all observers, and the figures listed in this survey show this same gradual increase in haemoglobin concentration in school-children as age progresses and, since the vast preponderance of children in the two higher age-groups were males,

this rise is continued through and after puberty. The explanation of this is difficult. Possibly the exanthemata of childhood occurring mainly in the lower age-groups and the gradual weeding out of the unfit and the building up of immunity to other diseases in the remainder account for this. Another possibility is malnutrition in the lower age-groups from the traditional methods of feeding adopted by the general public, which favour a diet for younger children in which carbohydrates take the main place at the expense of protective foods.

Race.

The figures for Europeans and Coloured are shown separately, not because race *per se* is expected to alter the haemoglobin concentrations, but because the Coloured children as a group can be stated definitely to be undernourished either in the quantity or quality of their diets or in both. Diagram I shows graphically the difference between European and Coloured school-children under various conditions, as regards their nutritional state, which was assessed from the general clinical condition at routine medical inspection in accordance with the Dunfermline classification: A = Excellent, B = Normal, C = Subnormal, D = Bad.

Whereas the European child's diet may often be a badly balanced one, it can be taken as exceptional for this to be deficient in quantity. In the case of the Coloured child, it is probably exceptional for him to receive a well-balanced diet, and this rule applies even to the more well-to-do Indians, while there is good reason to believe that most Coloured children do not receive an adequate quantity of any food in their homes on account of poverty.

In addition to a defective diet, the conditions under which the Coloured child is reared often tend to lower his physical state. The standards of housing, hygiene and sanitation adopted by the Coloured population are generally much below those of Europeans. The Coloured children may therefore be taken as representative of a group showing widespread malnutrition due to a defective diet and a lower standard of living. On the other hand, if climate has any appreciable deleterious influence on the physical health of the European in this Colony, it is possible that the heredity of the Coloured child may confer on him some advantage in this respect, but this is mere conjecture.

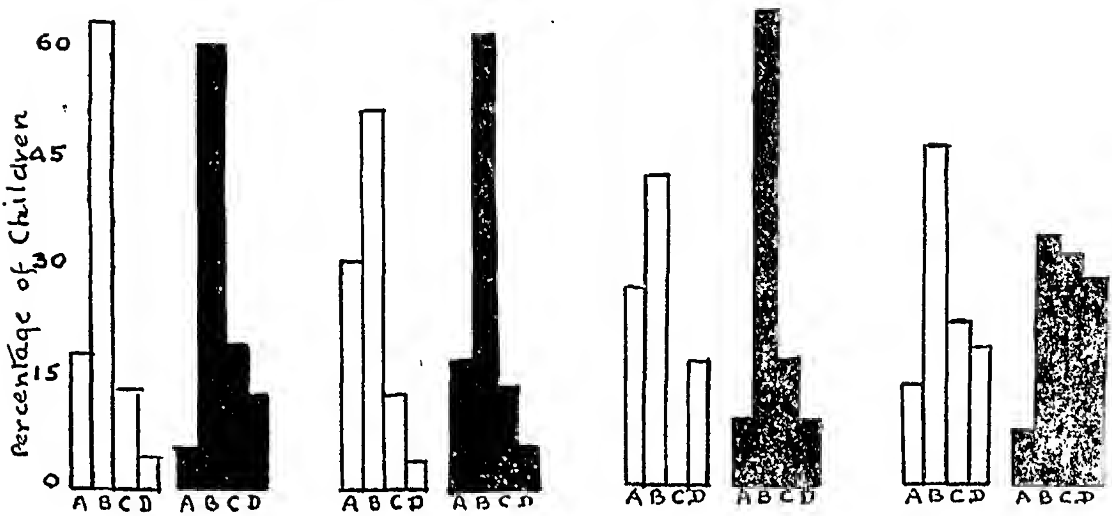
Parasitic Infection.

The only evidence accepted in this survey of parasitic infection was the presence of helminthic ova in urine or stool, or the presence of parasites in the blood smear or a palpable spleen. The finding of a high eosinophil count is not considered sufficient evidence, in the state of present knowledge, to justify the assumption of parasitic infection. The writer's figures of a survey in 1938 showed that helminthic infections in this Colony, other than schistosomiasis, have a relatively negligible effect on the eosinophil count, possibly because the

DIAGRAM I.

Nutritional standards of school-children in Southern Rhodesia compared under various conditions.

Europeans : unshaded.
Coloured : shaded.
Nutritional standards. A = " excellent."
B = " normal."
C = " subnormal."
D = " bad."



(1) All children examined in 1938.

Numbers :
Europeans : 4,582
Coloured : 598

Percentage :

Europeans :

A. ...	18·41
B. ...	63·98
C. ...	13·57
D. ...	4·04

Coloured :

A. ...	5·69
B. ...	60·20
C. ...	20·73
D. ...	13·38

(2) Boarders not proved to have parasitic infection.

Numbers :
Europeans : 1,163
Coloured : 508

Percentage :

Europeans :

A. ...	31·40
B. ...	52·19
C. ...	13·32
D. ...	3·35

Coloured :

A. ...	17·71
B. ...	62·59
C. ...	14·77
D. ...	4·92

(3) Boarders proved to have parasitic infection.

Numbers :
Europeans : 100
Coloured : 45

Percentage :

Europeans :

A. ...	27
B. ...	42
C. ...	14
D. ...	17

Coloured :

A. ...	8·89
B. ...	64·45
C. ...	17·78
D. ...	8·89

(4) Day - Scholars proved to have parasitic infection.

Numbers :
Europeans : 156
Coloured : 60

Percentage :

Europeans :

A. ...	13·46
B. ...	45·52
C. ...	22·44
D. ...	18·58

Coloured :

A. ...	6·67
B. ...	33·33
C. ...	31·66
D. ...	28·33

infections are mild ones, while many cases showing schistosome ova in the urine or stool have an eosinophil count of under 5 per cent. and others showing no ova have eosinophil counts ranging up to 85 per cent. It was pointed out, that those with ova showing low counts might be late cases with well developed immunity, while those showing no ova but high counts might be early cases. Investigations are still proceeding to solve this problem, but until a solution has been reached it is felt that it is inadvisable to diagnose parasitic infection in individuals on the grounds of a high eosinophil count.

The fact that no parasites or ova were found in the specimens from any child by no means excludes the possibility of parasitic infection, so it is therefore impossible to draw up a list of parasite-free children for comparison with the parasite-infected which have therefore to be compared with the whole group. Hundreds of children were seen during this investigation who reported frequent attacks of fever, or who had just recovered from an attack, yet, since they showed no objective evidence of malaria, they were not included in the parasite-infected group; many of these children showed low Hb indices, particularly those reporting a recent attack, so it is highly probable that these were all malarial cases. This has tended to lower the average figures of the main groups considerably.

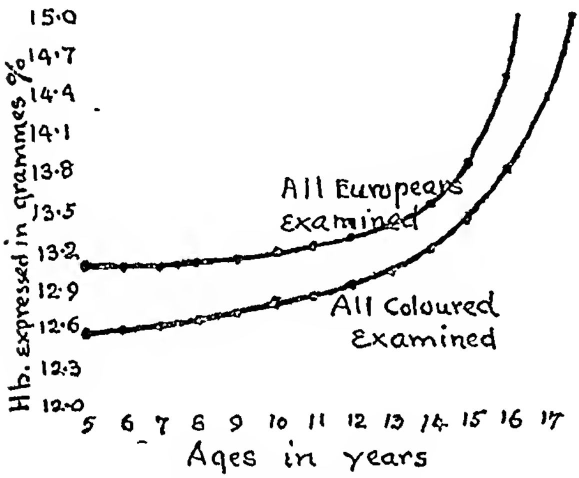


DIAGRAM 2.

Average Hb indices of school-children in Southern Rhodesia in 1939.

For figures see Tables I and II.

The effect of parasitic infection on the Hb index varies considerably with such factors as intensity, efficiency of treatment, immunity and diet, so it would not be expected that the haemoglobin indices of parasite-infected children would show individually any constant or dramatic difference. However, the figures for the parasite-infected groups in Tables III and IV are generally lower than those of the whole groups of each race, and the proportions of anaemic children are definitely greater. The effect is greater among the Coloured children, probably from the combined effect of malnutrition and lack of treatment and more intense infection, while the effect is less marked on the European boarders in Table VII on account of the better nutritional state (see Diagram 1) of boarders, more efficient treatment and less intense infections, since, with one or two exceptions, the boarding hostels do not offer many chances of endemic infection to the boarders who thus have a respite during term-time from the conditions encountered in their own homes.

Figures showing the respective effects of chronic malaria (palpable spleen) and hookworm infection on the haemoglobin index were compiled by the

writer in 1938. Of 133 children with palpable spleens, eighty-three had an index of 60 per cent. (Tallqvist) or less, and the average index was 61·73 per cent. with a standard deviation of 9·7. Of twenty-nine children with hookworm infection, thirteen had an index of 60 per cent. or less and the average index was 65·9 with a standard deviation of 8·1.

BLACKIE (1936) published figures of the haematological findings of ten cases of urinary schistosomiasis. The haemoglobin indices of these cases ranged between 9·8 grammes and 14·6 grammes Hb per 100 c.c. blood, with an average of 12·8 grammes (NEWCOMER). The same observer records the usual haemoglobin index of chronic malarial patients in the Colony as 10·8 grammes per cent.

The main endemic parasitic infections, *i.e.*, malaria, schistosomiasis and hookworm can therefore be stated to have usually a lowering effect on the haemoglobin index, and in view of the insidiousness of these conditions and the widespread and inestimable prevalence of the first two mentioned, it is practically certain that the normal standard adopted in this survey is well below the normal standard of healthy persons living under ideal conditions in this Colony. Further intensive investigation is required to determine the real normal standard.

Climate.

No evidence is yet forthcoming that the altitude of the plateau region of this Colony has any effect in raising the haemoglobin index. The presence of a multitude of anaemia-producing factors which cannot be discounted without repeated investigations renders it difficult to determine the normal standard of healthy persons. However, the fact that 371 (17·1 per cent.) of the European children showed haemoglobin indices above the adopted normal standard ranging from 15 to 19·2 grammes Hb per 100 c.c., suggests that the effect of altitude is masked in the majority of cases by anaemia-producing influences which give rise to that Rhodesian " tiredness " which disappears so rapidly with a change to sea level.

The effect of the summer heat on the blood, suggested by BLACKIE, is not evident in the figures listed in this survey. MATSUNOBU (1936), working in Taihoku, compared the erythrocyte count of eleven healthy men and twenty-three women in the winter, and ten men and twenty-three women in the hot, moist summer, and found no difference. Unfortunately, he did not mention haemoglobin estimations. However, it is felt that this reference to the summer heat of Southern Rhodesia gives rise to an exaggerated conception in so far as it applies to the plateau region. Compare, for instance, the mean maximum temperature, 85·2° F., and the mean minimum, 57·8° F., during the hottest months of the year on the Southern Rhodesian plateau with the respective maximum 97·5° F. and minimum of 81·5° F. at Lahore. Without taking

into account the lower humidity and barometric pressure of the Rhodesian atmosphere, the temperatures alone cannot be considered in the same stratum as those pertaining to most other parts of the tropics and sub-tropics. CASTELLANI (1931), while admitting that a tropical humid climate may induce in Caucasians a slight decrease in the amount of haemoglobin, quotes WINTROBE and MILLER's figures of 100 young men and 50 young women residing in Louisiana, and says that the results of these blood determinations seem to indicate that the hypothesis that a physiological anaemia exists in tropical and sub-tropical climates is without foundation in fact.

The facts that fans or punkahs are unknown in private residences in Southern Rhodesia, that a cloth suit is comfortable throughout the year, that one or more woollen blankets are necessary every night and that active games and sports are indulged in during the middle of the day throughout the year, speak for themselves.

However, account should be taken of the fact that whereas malaria and other parasitic infections are more commonly contracted during the summer months, which should tend to lower the Hb index, nevertheless, this is also the rainy season when crops and local foodstuffs are most abundant, and this should tend to counteract the effect of parasitic infection in the same way that it tends to reduce the incidence of deficiency states. This is a possible explanation of the fact that anaemia was no more prevalent among the Europeans examined in the summer months; whereas the proportion of parasitic infection, although not appreciably higher as gauged by the figures listed as proved cases, was undoubtedly higher in fact from the abundance of unproved and therefore unlisted cases which tend to produce greater anaemia than the listed chronic cases.

School Boarding Hostels.

Whereas a survey of the dietaries in school boarding hostels during the year concerned did not reveal ideal conditions, it is believed that, on the whole, European boarders receive a more balanced dietary than in their own homes, and Coloured boarders receive a far better dietary altogether.

Apart from the dietetic aspect, the generally more hygienic environment of most of these hostels, the more orderly routine of meals, sleep, work and athletics and the lessened exposure to parasitic infection, as compared with the boarders' homes, which are mainly in rural districts or northern or eastern territories, and regular, free medical attention should tend towards better health. This seems to be the case as shown by routine medical inspection (see Diagram I).

European boarders show a somewhat higher incidence of chronic parasitic infections, doubtless because of their greater exposure to these in their rural or extra-territorial homes, but these appear to be mild in their effect, judging by their negligible effect on the haemoglobin concentration and the general

condition of the children. These mild, insidious, parasitic infections are usually symptomless, hence they are seldom detected without a routine medical and laboratory examination. Parasitic infections of greater intensity would lead to symptoms and efficient medical treatment, hence the absence of low Hb indices in conjunction with parasitic infection among European boarders.

In the case of the Coloured boarders, it will be observed that only 90 were examined in this survey. These were all in one sehool adjacent to the Kalahari Desert and had only returned to school two weeks previous to being examined. The time of the examination was the driest part of the year when protective foodstuffs would be unobtainable in these children's homes. Incidentally, three of these children had definite pellagra and two others had suspicious signs of this deficiency state. Although parasitic infections are less common in this district than in the rest of the Colony, owing to its dryness, malnutrition and deficiency states are prevalent, and at the time of the examination these children had not returned to school from their homes for a long enough period for their state of malnutrition to disappear, hence their haemoglobin indices were on the whole slightly lower than those of the main group of Coloured children in Table II. It is probable that had they been examined some weeks later in the term the effect of the better dietary and environment in the boarding hostel would have been more obvious.

In connection with the survey of dietaries in school boarding hostels, comparisons were made of the dietaries as regards the average protein and iron intake in proportion to the ages and sexes of the boarders subsisting thereon. When Hb examinations were made of these boarders, sub-groups of these were compared in respect of a high or a low protein or iron intake, as gauged by the average intake (in proportion to age and sex) of all the dietaries. Of 189 boarders receiving a relatively high protein intake, 19 (10·1 per cent.) were anaemic, while of 312 on a lower protein intake, 58 (18·6 per cent.) were anaemic. Of 307 boarders on a relatively high iron intake 41 (13·4 per cent.) were anaemic, while of 194 on a lower iron intake 36 (18·6 per cent.) were anaemic.

On account of the multiplicity of factors in diet alone which may influence the haematological state, it is not considered that these comparisons in respect of single components of a diet are of much significance in the absence of controlled experiments with the elimination of all other attributes in respect of diet or disease which might influence unevenly two groups which are being compared in respect of a single attribute.

Summary.

(1) A survey of the haemoglobin indices of 2,173 European and 304 Coloured school-children, resident on the plateau region of Southern Rhodesia, was completed in 1939 by Newcomer's method. Of these children, 320 Europeans (14·7 per cent.) and 70 Coloured (23 per cent.) were found to have sub-clinical anaemia.

(2) A provisional normal standard of 13·49 grammes Hb per 100 c.c. of blood was adopted and a range of normality between 12 and 14·9 grammes, but it is considered that this standard is probably below that of healthy persons living under ideal conditions in this Colony.

(3) The effects of age, race, parasitic infection, climate, diet and residence in a boarding hostel on the haemoglobin index have been studied and, while it is considered that all these have either a direct or indirect influence, the main factors which lower the haemoglobin index directly are (*a*) faulty diet, and (*b*) parasitic infection. The relative effects of these two can only be gauged by controlled experiment and they are both remediable. The climate is not considered to have any direct deleterious effect on the health of Europeans, and this contention seems to be supported by the fact that the physical health of the European child appears to be on the whole at least as good as in Europe, in spite of the diseases and dietetic errors common to Europe, with the additional prevalence of parasitic diseases and, it is alleged, a local, relative, dietetic deficiency in minerals.

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OBSERVATIONS ON THE FACIAL APPEARANCE IN CASES OF BILHARZIASIS.

BY

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Medical Superintendent, Mtemwa Leprosy Hospital, Southern Rhodesia.

My work on this subject originated 8 months ago when, at Mtoko Clinic, I happened to notice a peculiar facial appearance in many young natives who were attending the clinic for treatment for bilharziasis. The exact appearance is not easy to describe—photographs show it more clearly—but briefly, it is a thinning and loss of subcutaneous fat in the region of the horizontal ramus of the mandible. This wasting is very definitely localized—there is no hollowing of the cheeks—and it gives the face, when viewed full-face, a characteristic appearance. From in front of the ears the face is straight, down to a point in front of the angle of the jaw: thence to the chin, a triangular appearance is seen; or even an indrawing of the tissues about the middle of the lower jaw. This can best be shown by the simple diagram below.

Normal.

Bilharzia "Face."

Another worker has also described a facial appearance in bilharziasis, but this seems quite different. In his own words, it is

"an in-drawing of the cheeks. They tend to become sunken as a result of which the malar bones appear prominent. The face tends to become on the whole more triangular. I explained this as due to chronic wasting, the earliest area to show this being in the face. Not all, however, have this appearance and there are many who do not show this at all."

In my own opinion, this appearance, which I have often noted myself, is a very much later stage of the appearance I have here described. Of the number of

* Since writing this paper the author, Dr. G. B. Davis, has died suddenly of cerebral malaria.

cases who harbour bilharzia there cannot be many who show this sign of advanced wasting, but a very large percentage do show the face that is characteristic of very early wasting, the " bilharzia face " which I have described.

At first I thought that this appearance could be considered definitely diagnostic of bilharziasis : I also thought that it was of very great practical importance and might be applied practically with very successful results. I have had to modify these ideas considerably. To prove or disprove the genuineness of my observations, I proceeded on the following lines :—

1 2 3 4

FACIAL APPEARANCE IN BILHARZIASIS
FIG. 1. Normal face and " bilharzia face."
FIGS. 2, 3 and 4. "Bilharzia face."

I visited kraal schools every day for several weeks. At any particular school between 100 and 300 young natives were gathered together, and from each I took the name and kraal, inquired into any history of haematuria, felt for an enlarged spleen, and took a specimen of the urine. All these were examined at the Public Health Laboratory for ova : this was rather laborious but resulted in my obtaining a series of about 2,000 cases. This series was analysed, and

as a final check I visited one more school and made an impartial survey of 180 young natives and analysed this series. The figures of these analyses are attached and, to a certain extent, they speak for themselves. It will be noticed that in the whole series young natives from the age of 3 to 18 years were all

TWO BILHARZIA INVESTIGATION AT ALL SOUL'S MISSION.

FACIAL APPEARANCES AND SPECIMENS OF URINE.

I

162 *Specimens Examined.*

Positive face and positive specimen	94	
Negative face and negative specimen	12	
Total ...	106	" correct."
Positive face and negative specimen	15	
Negative face and positive specimen	41	
Total ...	56	" incorrect."

Deducting from these 56, the 29 patients either over 14 or under 5 years of age, the true number " incorrect " is 27.

Expressed as percentages of the 162 examined the figures are : correct results = 83·4 per cent. ; incorrect results = 16·6 per cent.

II

1,006 *Specimens Examined.*

Positive face and positive specimen	622	
Negative face and negative specimen	52	
Total ...	674	" correct."
Positive face and negative specimen	70	
Negative face and positive specimen	262	
	332	" incorrect."

As percentages of 1,006 examined correct results = 67 per cent. : incorrect results = 33 per cent.

(In this series the age of the patient was not taken into account.)

examined impartially. After working out the results, I found they were very different from my original expectations. My conclusions are given later, but it can be said here that the percentage of correct results is quite high ; by " correct results " is meant either the presence of the bilharzia face together with ova in the urine, or the absence of the face and the absence of ova.

The main criticism that can be levelled at these figures is, it seems to me, that if bilharziasis is so prevalent amongst the natives in this district even the facial appearance may be merely a coincidence. I do not believe this to be the case as I can show later. There are, however, probable explanations for some of the discrepancies in these figures. By " discrepancies " is meant the presence of the bilharzia face and the absence of ova, or the absence of the " face " and the presence of ova. In quite a large percentage of the cases who show no face and yet have ova, the age of the children was below 4 or over 14 years. In the chubby face of a native child under 4 years it is very difficult to see wasting. Again, in the case of an adolescent boy of 15, he is already

beginning to develop his natural adult facial appearance and to lose naturally his youthful facial fat. Therefore in cases in which I thought I observed the bilharzia face and no ova were found in the urine, it is possible that (1) repeated specimens would have shown ova; (2) as actually happened several times, the case had been treated and the face had not had time to return to normal; (3) in the very earliest stage of this wasting it is almost impossible to decide one way or another, and rather than leave the question of the face as "query," I decided to designate one way or the other.

Conclusion and Results.

The conclusion, therefore, that I have come to is that young natives who are suffering from bilharziasis have very often a definite facial appearance. It is not denied that they may be suffering from some other chronic disease as well as or apart from bilharziasis. The observation can only apply to young natives because the older ones develop their natural characteristic face and it becomes impossible to see any pathological change in it.

This investigation has brought to light the enormous amount of bilharziasis in this district alone. Of about 2,000 natives that I examined some 90 per cent. were infected. This stimulated me to organize treatment and at present I am giving personally over 1,000 intravenous injections of sodium antimony tartrate. My earlier expectations were that it would be possible to line up a given number of natives and decide by merely looking at them which were infected and which were not. If this were done without examining specimens of urine, a large number would either be missed or else treated unnecessarily. A "bilharzia face," therefore, cannot be taken alone as a means of diagnosis.

The value of the observations, in my opinion, is that this "bilharzia face" can be a very useful aid to diagnosis. Every day at the clinic several cases come to me, complaining of vague symptoms suggestive of bilharziasis: the facial appearance often confirms diagnosis of this and treatment is instituted forthwith. Again, on many occasions, cases have come to me with some trivial complaint—toothache, for instance: I have noted a suggestive facial appearance and then inquired whether there had been any history of haematuria. Nearly every time such a history has been elicited from the patient. The average native rarely comes for treatment for bilharziasis. In fact, in my series only 2 per cent. had voluntarily come forward for treatment. This is due, I think, to the prevalence of the disease, to the ignorance on the native's part of the abnormality of haematuria, and to their ignorance of the fact that bilharziasis can be effectively treated.

So by noting the facial appearance of a patient who attends for some other complaint one may be at once stimulated to inquire about a possible history of bilharziasis and in this way many cases are found and treated that would otherwise never have been discovered.

CORRESPONDENCE.

THE DIAGNOSIS OF BILHARZIASIS IN SOUTHERN RHODESIA.

To the Editor TRANSACTIONS *of the Royal Society of Tropical Medicine and Hygiene.*

SIR,

In a recent number of these TRANSACTIONS* Dr. MICHAEL GELFAND wisely noted the value of giving antimony injections in cases of suspected bilharzia infection, for a patient may harbour non-laying schistosomes such as undeveloped forms and adult males, or the escaping ova may be scarce even if the precaution is taken of securing the last portion of urine which can be expressed.

A specimen containing few ova does not always contain albumin (which can be revealed by the cold nitric acid test); and I have found numerous ova in the centrifugalized deposit of urine when the hospital chart stated that the urine was free from sugar or albumin, thus excluding some other conditions.

I am, etc.,

F. GORDON CAWSTON.

TEMPERATURES OF ANIMALS.

To the Editor, TRANSACTIONS *of the Royal Society of Tropical Medicine and Hygiene.*

SIR,

In No. 4, Volume XXXV, of the TRANSACTIONS, there is a letter from Mr. H. E. HORNBY concerning the "temperaturing" of animals. It seems unfortunate that Mr. HORNBY did not make his criticisms when I forwarded my article, on "the relation between the virulence of *Trypanosoma rhodesiense* towards rats and the normal blood temperature of its previous mammalian host," to him for his approval prior to submitting it to you, when I could have pointed out and inserted that:—

(1) All the temperatures were taken before 7 a.m. daily and the air temperature was seldom above 23° C. (73° F.).

(2) The data have been tested by statistical methods and each group shows (*a*) Normal distribution curves. (*b*) There are no significant differences between the mean temperatures of each individual animal of the same species ("t" test and "P" taken as 1 in 100). (*c*) There are significant differences between the mean temperature of each of the various species of animals listed ("t" test and "P" taken as 1 in 100).

(3) Although the normal blood temperature of an animal is affected by the climatic conditions, each individual of the species is affected in the same way; and, since the body temperatures for each species have been determined in the same place and time as the other observations on the virulence of *T. rhodesiense*, the correlation between them should hold.

(4) In paragraph 2 of Mr. HORNBY's letter, he states that "many more observations are necessary before comparative figures like those in Column 3

* GELFAND, MICHAEL. (1942). The diagnosis of kala-azar in Southern Rhodesia. *Trans. R. Soc. trop. Med.*, 35, 281.

of Table II of his article can be accepted." Since these figures have been submitted to statistical treatment by accepted methods (see FISHER*) Mr. HORNBY has entirely ignored these.

(5) Comparing Mr. HORNBY's statement that the expected early morning temperature of a healthy sheep at Mpwapwa is 102° F. (39° C.) and the mean temperature of sheep in Europe is given as 40° C. (104° F.) in STARLING'S *Principles of Human Physiology*, 6th edition, page 1010, it would appear that the sheep at Mwapwa are even more abnormal than the samples taken by me at Tinde: the latter showed a mean temperature of 103·4° F.

(6) The original data are still available and copies can be sent to anyone who may care to examine them.

I am, etc.,

F. L. VANDERPLANK.

* R. A. FISHER, *Statistical Methods for Research Workers*. 6th edition. London and Edinburgh: Oliver & Boyd.

TREATMENT OF MALARIA.

To the Editor, TRANSACTIONS *of the Royal Society of Tropical Medicine and Hygiene.*

SIR,

In his reference to the Quinine-Mepacrine-Pamaquin treatment of malaria as a "blunderbuss" treatment, Professor YORKE, at the meeting of the Society on 23rd July, asked "Why the pamaquin?" but as he referred to it as "the Army treatment" I did not feel that my entry into the fray was indicated.

Surely Professor YORKE must be aware of the opinion expressed in the *Fourth General Report of the Malaria Commission of the League of Nations* (1937) that "Plasmoquine has a definite effect upon the frequency of relapses of benign tertian or quartan. In association with quinine or atebrin, or administered after either of these two drugs, it is to a marked degree effective in preventing relapses in benign tertian (except perhaps in the case of a few particular strains) and quartan, and appears similarly to reduce the frequency of malignant tertian relapses. . . . Consecutive treatment with atebrin first and then with plasmoquine . . . diminishes substantially the number of relapses both in malignant tertian and, more especially, in benign tertian and quartan" malaria.

In endemic areas, where admittedly it is often impossible to distinguish between a relapse and a fresh infection, it is not easy to assess the value of plasmoquine as an "anti-relapse" drug, but it is widely used as such, its value being, perhaps, based rather on clinical impressions than on statistics.

Working in this country, Professor YORKE may be better able to assess this "anti-relapse" value and his opinion therefore carries all the more weight, but after using the "blunderbuss" treatment for some years I feel sure that each barrel has its own special purpose and that the discharge of the three barrels consecutively has a "broadside" effect that is not obtained by one or two of them alone.

I am, etc.,

F. E. LIPSCOMB,

Acting Group Captain, Royal Air Force.

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TRANSACTIONS
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COMMUNICATIONS.

SPRUE

BY

HUGH S. STANNUS, M.D., PH.D., F.R.C.P.
London.

1. During the past two or three years several articles have appeared dealing, from various points of view, with sprue and other possibly allied conditions in which steatorrhoea is a prominent symptom. Since there is no general agreement between the views expressed and since no one of the authors gives anything more than a tentative explanation of the facts, no apology is needed for also entering this speculative field. In this paper I shall only deal with sprue, though of course references will be made, in passing, to non-tropical sprue or idiopathic steatorrhoea and coeliac disease. In the first place I cannot help feeling that the moment has not yet arrived when any classification of these several conditions can be made; attempts to introduce new terminologies are to be deprecated. There is no advantage to be gained by the use of such terms as "Gee-Thaysen disease" and "Gee-Herter disease" as advocated by O'SULLIVAN and MOORE (1941). We all know what we mean by sprue, idiopathic steatorrhoea and coeliac disease; surely it is better to wait until further research reveals their true pathogeny before attempting their better classification.

2. I would like also to take this opportunity of correcting one or two statements concerning pellagra made by recent writers on sprue. HURST (1942), in referring to the occurrence of sprue in this country, adds: "It has gradually become recognized that true beriberi is seen in England as well as in hot climates, wherever the essential dietetic deficiency is present, and the same is true about pellagra which was long recognized as a purely tropical disease." MANSON-BAHR (1941) rather suggests the same thing when he remarks that sprue and pellagra "are apt to occur in the same countries." Again the writer of an annotation on "the sprue syndrome" in the *British Medical Journal* (1941), says, "as is

well known, this [pellagra] is a comparatively common disease of tropical and subtropical countries," adding: "In its geographical distribution pellagra frequently overlaps sprue," citing as his authorities for these statements WOOD (1925) and MANNING (1909). But MANNING described a condition in Barbados which, though he called it "psilosis pigmentosa," was certainly not sprue, whether it was pellagra seems uncertain. It is true the term psilosis has been used for sprue then referring, as does the word sprue itself, to the "stripping" of the tongue. MANNING used the word in referring to the "stripping" of the skin over haemorrhagic areas.

In the case of WOOD, no mention is actually made of cases of sprue and pellagra in the same area, he was speaking of the differential diagnosis of pellagra which was then occupying his attention in Carolina.

The facts are that there is no correlation between the geographical distribution of the two diseases and that pellagra has never been considered as a tropical disease. Is it necessary to recall the fact that pellagra was first described in Spain, then in Italy, followed by France, later in south-east Europe and Egypt, etc. ? There are several other statements in the annotation above mentioned which are open to question.

3. The first article which calls for discussion is one by IZOD BENNETT and HARDWICK (1940), entitled "Chronic Jejuno-ileal Insufficiency." In this paper the authors group together sprue and allied conditions as "due to the same cause—*i.e.*, chronic jejuno-ileal insufficiency with consequent defects of secretion and absorption," suggesting at the same time that the condition is reproduced when the total function of the small intestine is put out of action as by surgical intervention. They include in their complex of symptoms steatorrhoea, with fully split fat, tetany, macrocytic anaemia, rickets and stunting (in children), stomatitis and sore tongue, skin changes resembling pellagra, megacolon, low blood values for calcium, phosphorus and nitrogen, achlorhydria and a flattened glucose curve.

To these they add "typical carbohydrate dyspepsia with gassy distension of the bowel owing to bacterial hydrolysis of starch following the failure of amylolytic digestion by succus entericus." That some, or even many, of these symptoms may be met with in sprue and the rest of the group is surely well recognized, but when these authors state "Nevertheless we feel that up to now there has been a general failure to recognize that there is present something far more extensive than a mere inability to absorb fat and fat-soluble substances," then they touch on a matter concerning which there may be a difference of opinion. BENNETT and HARDWICK suggest that the "insufficiency" affects not only absorption but also secretion and that it may be total.

In sprue this is certainly not the case, the secretory activity of the small bowel and its glandular appendages is, as far as is known, normal; muscle fibre is digested, fat is split and, contrary to the statement of these authors,

there is no failure of starch digestion ; as remarked by HURST and KNOTT (1931), there is no sign of intestinal starch dyspepsia.

The same appears to be true of coeliac disease. BAUR (1928) found by duodenal tubage that the secretion of bile, lipase, trypsin and amylase to be the same as in the normal child.

This hypothesis of BENNETT and HARDWICK, therefore, one feels rests on no basis of fact ; moreover, no explanation is forthcoming concerning the cause of the " insufficiency " which might help us to understand the pathogeny of the conditions they discuss.

4. A more recent paper on the causation of " the sprue syndrome " is by HURST (1942), of which a summary appeared in a letter to the *British Medical Journal* (HURST, 1941).

He believes that tropical sprue, non-tropical sprue (or idiopathic steatorrhoea) and coeliac disease are varieties of the same disorder, differing only in the part of the world in which the disorder originated and in the age of the patient. He says, " There are three characteristic and constant features of the syndrome : (*a*) the stools contain excess of split fat but no excess of neutral fat, meat fibres or starch and no inflammatory material ; (*b*) radiography demonstrates the disappearance of the normal feathery or herring-bone aspect of the duodenum and jejunum produced by the valvulae conniventes ; (*c*) no pathological changes are found in the intestine after death if postmortem damage has not taken place. With adequate treatment normal absorption of fat is restored together with the normal radiographic appearance of the small intestine."

He suggests " that the characteristic features of the " sprue syndrome " are the result of paralysis of the muscularis mucosae which would lead to the loss of the pumping action of the villi, by means of which fat is conveyed from the lacteal radicles of the villi into the larger lacteals, and to flattening of the valvulae conniventes without changes in the normal appearance of the mucous membrane. Paralysis of the muscularis mucosae may be secondary to loss of the normal stimulant of Meissner's (submucosal) plexus or to the effect of vitamin deficiency or some toxaemia on the plexus."

HURST'S paper and the theory he has put forward are based largely on the writings of VERZÁR and his colleagues—Absorption from the Intestine (1936), and upon the radiological observations described by GOLDEN of New York (1941).

5. In regard to HURST'S third point, this is now generally agreed, though MANSON-BAHR (1939), in defining sprue, says it " manifests itself by a catarrhal inflammation of the whole of the intestinal tract," and the writer of the annotation in the *British Medical Journal* (1941) concludes by stating " but on microscopical examination there is usually evidence of chronic inflammation." Again, THOMPSON (1940) speaks of atrophy of the gut and ulceration in the small and large intestine and " papillary regeneration " following treatment. Even GOLDEN (1941) refers to the pathological changes found at necropsy.

well known, this [pellagra] is a comparatively common disease of tropical and subtropical countries," adding: "In its geographical distribution pellagra frequently overlaps sprue," citing as his authorities for these statements WOOD (1925) and MANNING (1909). But MANNING described a condition in Barbados which, though he called it "psilosis pigmentosa," was certainly not sprue, whether it was pellagra seems uncertain. It is true the term psilosis has been used for sprue then referring, as does the word sprue itself, to the "stripping" of the tongue. MANNING used the word in referring to the "stripping" of the skin over haemorrhagic areas.

In the case of WOOD, no mention is actually made of cases of sprue and pellagra in the same area, he was speaking of the differential diagnosis of pellagra which was then occupying his attention in Carolina.

The facts are that there is no correlation between the geographical distribution of the two diseases and that pellagra has never been considered as a tropical disease. Is it necessary to recall the fact that pellagra was first described in Spain, then in Italy, followed by France, later in south-east Europe and Egypt, etc.? There are several other statements in the annotation above mentioned which are open to question.

3. The first article which calls for discussion is one by IZOD BENNETT and HARDWICK (1940), entitled "Chronic Jejuno-ileal Insufficiency." In this paper the authors group together sprue and allied conditions as "due to the same cause—*i.e.*, chronic jejuno-ileal insufficiency with consequent defects of secretion and absorption," suggesting at the same time that the condition is reproduced when the total function of the small intestine is put out of action as by surgical intervention. They include in their complex of symptoms steatorrhoea, with fully split fat, tetany, macrocytic anaemia, rickets and stunting (in children), stomatitis and sore tongue, skin changes resembling pellagra, megacolon, low blood values for calcium, phosphorus and nitrogen, achlorhydria and a flattened glucose curve.

To these they add "typical carbohydrate dyspepsia with gassy distension of the bowel owing to bacterial hydrolysis of starch following the failure of amylolytic digestion by succus entericus." That some, or even many, of these symptoms may be met with in sprue and the rest of the group is surely well recognized, but when these authors state "Nevertheless we feel that up to now there has been a general failure to recognize that there is present something far more extensive than a mere inability to absorb fat and fat-soluble substances," then they touch on a matter concerning which there may be a difference of opinion. BENNETT and HARDWICK suggest that the "insufficiency" affects not only absorption but also secretion and that it may be total.

In sprue this is certainly not the case, the secretory activity of the small bowel and its glandular appendages is, as far as is known, normal; muscle fibre is digested, fat is split and, contrary to the statement of these authors,

there is no failure of starch digestion ; as remarked by HURST and KNOTT (1931), there is no sign of intestinal starch dyspepsia.

The same appears to be true of coeliac disease. BAUR (1928) found by duodenal tubage that the secretion of bile, lipase, trypsin and amylase to be the same as in the normal child.

This hypothesis of BENNETT and HARDWICK, therefore, one feels rests on no basis of fact ; moreover, no explanation is forthcoming concerning the cause of the " insufficiency " which might help us to understand the pathogeny of the conditions they discuss.

4. A more recent paper on the causation of " the sprue syndrome " is by HURST (1942), of which a summary appeared in a letter to the *British Medical Journal* (HURST, 1941).

He believes that tropical sprue, non-tropical sprue (or idiopathic steatorrhoea) and coeliac disease are varieties of the same disorder, differing only in the part of the world in which the disorder originated and in the age of the patient. He says, " There are three characteristic and constant features of the syndrome : (*a*) the stools contain excess of split fat but no excess of neutral fat, meat fibres or starch and no inflammatory material ; (*b*) radiography demonstrates the disappearance of the normal feathery or herring-bone aspect of the duodenum and jejunum produced by the valvulae conniventes ; (*c*) no pathological changes are found in the intestine after death if postmortem damage has not taken place. With adequate treatment normal absorption of fat is restored together with the normal radiographic appearance of the small intestine."

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It is taking medicine a long time to emerge from a kind of " patholog stone-age " and to realize that postmortem room findings may have little bea on the derangements of function underlying the symptoms seen during lif

Those cases of gross disease in which a fatty diarrhoea occurs in some simulating that of sprue, will be considered later.

6. VERZÁR, upon whose writings both HURST and GOLDEN rely, belie that the neutral fat which finds its way into the lacteals was resynthesised the intestinal mucosa from fatty acid formed in the small intestine, that ph phorylation was an essential process for all fat absorption and that phosphor tion was in turn governed by an adrenal hormone. His thesis was based work performed on adrenalectomised rats. He believed that " In all cases disturbed or diminished activity of the adrenal cortex as in experimental avitaminosis, pellagra, tropical and non-tropical sprue and also in Addiso disease, disturbances of fat absorption have been seen."

VERZÁR's statement concerning Addison's disease has been challenged HURST, and it is certainly incorrect to suppose that steatorrhoea is characteris of pellagra.

BARNES and his colleagues (1939) and others have since shown by repeati VERZÁR's rat experiments that fat absorption is not disturbed by adrenalecto provided the animals are maintained in good condition by the administrati of adequate amounts of sodium chloride.

VERZÁR's main thesis has therefore been proved to be incorrect ; his theo of total fat splitting, phosphorylation and resynthesis to neutral fat, which accepted by HURST, appears to be fallacious, as will be shown later. Again, regard to the pumping action of the villi, failure of which is a main point HURST's proposition, no evidence is adduced to show that such a failure do in fact occur ; moreover, it has been shown by WELLS and JOHNSON (193 that the villi may be quite motionless during absorption and that the conver may be true. That the pumping action of the villi normally propels the conten of the lacteal radicles into the bigger lacteals cannot be doubted, but this one matter ; absorption by the epithelium lining the gut is another.

In view of what has been said above, my conclusion is that HURST's theo is lacking in support.

7. Next in regard to GOLDEN's article, embodying a lecture by a radiologi to radiologists. His material consisted of films selected because they showe the picture characteristic or suggestive of " the deficiency pattern " ; in fac the diagnosis of a deficiency state in the patient appears in many cases to ha rested on the X-ray picture. Abstracts of the patient's records were at th same time examined. The clinical diagnosis varied from hypoproteinaemia t acute appendicitis. " Eight cases showed sufficiently marked clinical signs t permit their classification in the group of recognized clinical entities, as sprue adult coeliac disease, etc."—a lengthy statement calculated to make anyone els

doubt the diagnosis. The pathological changes mentioned he has "summarized from necropsy reports of advanced (*sic*) human deficiency diseases such as sprue and beriberi."

He adds the postmortem findings in two fatal cases of uncertain diagnosis and a single biopsy specimen from a 61-year-old man stated to be suffering from non-tropical sprue but in whose case-notes there is no mention of steatorrhoea. The necropsy material I believe with HURST to be valueless. In the biopsy material degeneration of muscle fibres in the muscularis mucosae and of nerve cells in the myenteric and submucosal plexuses was discovered.

On this meagre evidence from a single case and without any observations on controls, GOLDEN has erected a theory postulating damage to the intramural nervous system of the intestine as the cause of the "deficiency mucosal pattern" and associates with this deficient absorption.

That changes in the mucosal pattern have been demonstrated in a variety of conditions is admitted, but that they all have a single common cause—"damage to the intramural nervous system of the intestine," as suggested by GOLDEN, is quite unconvincing. The facts upon which this theory and that of HURST are based can be interpreted, I believe, in another way. That the muscularis mucosae causes changes in the arrangement of the mucous membrane is unquestioned, emotion will initiate such changes, altering the mucosal pattern, but it is worthy of note that, as stated by WETZEL, in the infant there is but an insignificant development of mucosal folds, yet there is no deficient power of absorption.

GOLDEN makes a point of excepting pernicious anaemia from his deficiency group, while MACKIE and POUND (1935) include this disease with sprue, pellagra, beriberi and nutritional oedema as yielding rather similar radiographic pictures, analogous to those obtained in chronic ulcerative colitis though the parallelism is not exact.

8. As far as sprue is concerned I think the radiological findings of other previous workers in America are of more interest. The first observations were made by PILLAI and MURTHI (1931) but beyond a reference to motility they contain little. MACKIE (1933) described the X-ray appearances in a case of non-tropical sprue. The following year RHOADS and MILLER (1934) and MACKIE, MILLER and RHOADS (1935), carrying out investigations on sprue in Puerto Rico, chiefly from the point of view of the anaemia and its treatment by liver extract, demonstrated the distorted mucosal pattern in the duodenum, jejunum and also often in the ileum, with segmentation—signs which disappeared and reappeared with treatment and when treatment was discontinued for 10 to 14 days.

MACKIE, MILLER and RHOADS (1935) found the X-ray appearances in sprue to be characteristic—segmental distribution of the barium, either localized to one part or throughout the whole of the small intestine, the mucosal folds of the duodenum thickened and the lumen irregularly dilated; the valvulae

conniventes of the jejunum thickened, irregular in shape and more widely spaced, the lumen often dilated in isolated segments ; the barium passes through the jejunum slowly and irregularly, apt to collect in the dilated segments ; later all the barium may collect in a localized pocket ; the changes in the ileum are similar ; the colon may be dilated and redundant. After treatment evidence of recovery is seen.

SNELL and CAMP (1934) made similar observations in six cases of idiopathic steatorrhoea, when they pointed out the progressive nature of the changes in the small bowel, that delayed motility was a conspicuous feature and that the signs retrogressed with treatment. They thought " the intestinal phenomena were neither specific nor characteristic " and suggested that this would be explicable if vitamin deficiencies operated through the nervous system of the intestinal wall.

KANTOR (1939) gives a very good description of the radiological picture which, besides sprue, idiopathic steatorrhoea and coeliac disease, " may be the result of a variety of organic diseases that interfere seriously with the function of the lymphatics " in which a constant association with lack of fat absorption occurs. He mentions the loss of the " herring-bone " mucosal pattern in the jejunum, the apparent disappearance of the valvulae conniventes as if they had been " ironed out," the dilatation and segmentation of the small bowel, the delayed emptying and what he refers to as " the moulage sign," the appearance as if a wax model had been produced by the barium meal.

He points out, too, how these signs appear during periods of activity and disappear when the symptoms are quiescent.

9. To my mind KANTOR'S observations are the nearest to the truth when he remarks the association of the radiological picture he describes with interference with fat absorption. This picture, apart from sprue, idiopathic steatorrhoea and coeliac disease, may be reproduced in acute yellow atrophy of the liver, in jaundice due to obstruction of the bile duct, etc., and other conditions in which there is deficient fat absorption and the bowel contains in consequence a mass of fatty material. I have under my care at the moment a patient with an *aertrycke* infection who exhibits jaundice, steatorrhoea and an abnormal mucosal pattern.

The same picture again may be reproduced by " mesenteric block " with lacteal obstruction and the resulting steatorrhoea may in some cases closely simulate sprue, but the underlying cause is different.

Such cases have been reported by RYLE (1934) tuberculous mesenteric glands, FAIRLEY and MACKIE (1937) mesenteric gland lymphoma, etc. In these cases it is a kind of " back pressure " effect and not a primary defect of absorption and it seems possible that more complete investigation of cases may serve to differentiate the several clinical groups.

The after history of one of RYLE'S original cases has been published in an article by HURST, WRIGHT and RYLE (1942). It would appear at first sight to

oppose the hypothesis put forward by myself but more details would be necessary before discussing the case.

10. Thus far my own conclusions in regard to sprue are that the condition obtaining in the small intestine, as interpreted by radiological examination, is the expression of a passive reaction to its abnormal and bulky contents. There is a functional derangement of the bowel due to deficient fat absorption and not deficient fat absorption due to paralysis of the muscularis mucosae of its wall. This interpretation I believe receives confirmation from the fact that under certain conditions a similar derangement of the large bowel may occur, giving rise in coeliac disease to megacolon and in sprue to dilatation and redundancy as noted by MACKIE, MILLER and RHOADS but often not appreciated by writers on sprue, a point to be referred to again later.

HURST cites a case of calcified mesenteric glands with " the sprue syndrome " under the care of Professor WITTS in which the jejunum showed the normal feathery mucosal pattern in contrast to the smooth condition seen in cases with identical symptoms but no obstruction of the lacteals. From this single case, he argues, " The smooth contour is therefore not a result of the excess of unabsorbed fat in the intestine but must be the radiographic indications of the disappearance of the normal valvulae conniventes. Moreover, the changes are seen already in the duodenum where digestion of fat has hardly begun, so that there can be no question of the abnormal appearance being a result of the presence of excess of undigested fat."

This argument is, I believe, fallacious. It has never been shown that the abnormal mucosal pattern occurs in every case in which " the sprue syndrome " occurs unassociated with " mesenteric block." O'SULLIVAN and MOORE have recorded cases of idiopathic steatorrhoea with absence of this sign. The converse is true—abnormal mucosal pattern does occur in cases associated with obstruction of the lacteals as reported by GOLDEN. HURST says fat digestion has hardly begun in the duodenum but this is but a statement of opinion and probably incorrect. Though changes may be seen in the mucosal pattern of the duodenum by radiological examination, they are commonly most marked in the jejunum where the mass of fatty acid contents exerts its most marked effect according to my own view.

11. One of the main, if not the chief, consideration in sprue is the question of fat digestion and fat absorption, yet have we made little progress during the last 50 years towards solving the problem at issue—since, in fact, the time when it was first noted that sprue stools contained an excess of fatty material. We carry out a few estimations of the total fat content of dried faeces with the relative values for neutral fat and fatty acid, next a blood calcium estimation followed by a sugar tolerance curve and then rest satisfied with our biochemical inquiries, whereas these elementary laboratory tests hardly touch the fringe of the subject and indeed may lead to fallacious deductions. It does not even appear very certain what the normal figures for faecal fat may be, or how they

conniventes of the jejunum thickened, irregular in shape and more widely spaced, the lumen often dilated in isolated segments; the barium passes through the jejunum slowly and irregularly, apt to collect in the dilated segments; later all the barium may collect in a localized pocket; the changes in the ileum are similar; the colon may be dilated and redundant. After treatment evidence of recovery is seen.

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the observations of SHAPIRO and his colleagues (1936) upon which HANSMAN (1939) lays some emphasis. To a patient with a biliary fistula which could be opened or closed at will, they fed " deuterium labelled " fat. When the bile was allowed to flow to the exterior nearly all the supposedly ingested fat was found in the stools as fatty acid. It was, however, discovered actually that only 30 per cent. was " labelled " fat, 70 per cent. of the labelled fat had disappeared, presumably had been absorbed, while at the same time approximately the same quantity of unlabelled fatty acid had been secreted by the intestinal mucosa. If these observations do nothing more, they serve to show how complicated a process may be involved and further suggest how misleading may have been our interpretations in the past.

HANSMAN believes that fat absorption, secretion, desaturation and resorption are all processes bound up together, and that the fat appearing in the faeces, during starvation and after feeding, is made up of a remnant of the fat excreted by the bowel together with lipoid formed from epithelial debris and bacteria.

13. There appears to be some difference of opinion as to whether the excess of fat in the stools is due to the rapid passage of the contents through the bowel, " intestinal hurry " according to MANSON-BAHR (1941), or whether it is the presence of the fat in the bowel which causes diarrhoea.

BARKER and RHOADS (1937) attempted to settle this question and the question of absorption and excretion of fat in sprue. After a period of stabilization on a diet containing high protein content with fresh fruit, limited carbohydrate and practically no fat, five cases of tropical sprue were fed a meal of fat, 2 grammes per kilo body weight, consisting of butter and cream with bread as the vehicle. This was followed after an interval of from 12 to 48 hours by the passage of a bulky fatty stool. They argued that this lag period suggested that the results cannot be associated with a loss of fat due to excretion by the intestine but must be due to failure of absorption and that it was the presence of the fat in the bowel which caused the diarrhoea. We may agree with this interpretation but there is no proof. The facts as I see them suggest a delay in the passage of the fatty contents in the small intestine as revealed by radiological examination with no " intestinal hurry " and either rapid passage through the large bowel with diarrhoea or delay without diarrhoea according to the nature of the bowel contents, a matter which will be referred to again when discussing calcium.

14. Always remembering HANSMAN'S words, " it is useless making extravagant calculations on what we get out of the distal end if we do not know what goes in at the proximal end," the findings as generally stated in regard to sprue are in round figures :—

The weight of the faeces voided in 24 hours may be increased 5 to 10 fold. 50 to 60 per cent. of the ingested fat is found in the stool, 40 to 50 per cent. absorbed.

The weight of fat passed in the stool may vary from 15 to 65 grammes

should be expressed. The proportion of fat in the diet in any one day may vary widely, the changes undergone by ingested fat may differ with the quantity and nature of the fat and the composition of the rest of the diet.

When the lower limit for normal faecal fat is given as 10 per cent. and the upper limit as two and a half times that amount, it is obvious that the figures we obtain in sprue in the ordinary way can be but approximations.

Physiologists state that 50 per cent. by weight of faeces consists of the bodies of dead bacteria, composed in considerable part of lipoid material, a fact rather calculated to upset the comparison of ingested fat and faecal fat. Further, it appears to be generally assumed that, even in normal persons, the proportion of fat which is split remains constant. In sprue, arguing from the relative proportions of neutral fat and fatty acid it is often stated that splitting is normal or excessive, etc.

FAIRLEY (1936) states in regard to a series of cases in which faecal analyses were made " from the results it follows that in the majority of cases of tropical sprue fat splitting is normal ; in a few it is defective and in a few excessive." Such an argument I think may be open to doubt ; it presupposes that the stool findings are a measure of what has taken place in the small intestine, without regard to possible selective absorption for example.

To make this point clear, one may give what is perhaps an extreme illustration to show how a proportion of 1 : 2 in unsplit: split fat might be found on faecal analysis as the result of very different processes in the small bowel (stated in proportions) :—

20		20	
10 split of which 8 absorbed	10 unsplit of which 9 absorbed	16 split of which 14 absorbed	4 unsplit of which 3 absorbed
2 split :	1 unsplit	2 split :	1 unsplit
in faeces		in faeces	

This kind of possibility does not appear to have received any attention by writers on sprue. The variable factors influencing the amount of split fat in the stool, which are commonly not checked, include the amount of active lipase, the type of fat, the amount of soap formation, lipolysis by bacterial action, etc.

Other anomalies occur in reference to faecal analyses, for example, neutral fat has often been bracketed with fatty acid as unsoaped fat instead of considering together fatty acids and soaps and defining the nature of the soap, an important point when considering calcium metabolism in sprue.

12. The question of actual fat excretion by the bowel is a very difficult one and one which will not be discussed here, but it may be well to remember

in the mucous membrane of the small gut takes any part in the lipolysis within the lumen of the bowel. Normal splitting occurs in cases of obstruction of the bile duct, *i.e.*, in the absence of bile, but normally lipase forms adsorption complexes with calcium and bile salts which activate it in the alkaline medium of the upper part of the small intestine.

The addition of extra lipase to a known amount of ingested fat results in a marked decrease of the post-absorptive lipaemia proportional to the amount of extra lipase added; the fat is still absorbed from the intestine but diverted along some other path. This suggests that a greater proportion of fat has been split and that the fatty acid thus formed and subsequently absorbed is not resynthesized to neutral fat but goes to form other compounds as noted below.

The ingestion of neutral fat produces different results from those obtained with an equivalent amount of fatty acid and glycerol. If olive oil and oleic acid, both stained equally with sudan IV, are given with their food to two groups of rats for a period of 10 days, the animals receiving the glyceride have deeply stained fat depots, whereas those taking the acid show practically no depot staining. (Fat depots—collections of fat around kidney, in mesentery, skin, etc.). Faecal analyzes show that the fats are equally absorbed in both groups. From this it is deduced that the path followed by the glyceride is different from that followed by the fatty acid; in the case of the former the destination is the depot fat of the body, in the case of the fatty acid the destination is probably the liver as will be seen below.

In anaesthetized rats the lipids can be injected into the intestine and simultaneous systemic and portal vein specimens obtained. If glyceride is introduced, the systemic blood shows the normal increase of fatty particles and the lacteals appear milky but there is little change in the portal blood. If fatty acid and glycerol are injected, the portal specimens show a definite increase in the number of particles but the systemic blood does not and the lacteals do not become milky. Similar results have been obtained in human subjects fed by duodenal tube.

17. It therefore appears that only part of any ingested neutral fat undergoes splitting. This partial lipolysis may prove an important determining factor in fat metabolism. FRAZER (FRAZER, STEWART and SCHULMAN, 1942) believes that the unhydrolysed fat is absorbed as a fine emulsion. In the intestine there is an ideal system for emulsification. The fundamental factor being the formation of soap (stabilizer) *in situ* on the globules at the fat/water interface assisted by cholesterol complex formation. The absorption of this finely dispersed fat-in-water emulsion (the globules are probably of the order of $\frac{1}{2}$ μ diameter) by the epithelium lining the small intestine may be associated possibly in some way by phospholipid formation (FRAZER, unpublished communication). Of the experimental work that forms the basis of the hypothesis which predicates of fat preliminary lipolysis and subsequent resynthesis

in the 24 hours and may range up to a quarter of the total weight of dried faeces. Whereas the proportion of split to unsplit fat is normally as 2 : 1, in sprue the values may be as 3 : 1 or as high as 6 : 1.

In FAIRLEY'S (1936) series of seventy cases—in ten 50 per cent. or less was split, in eight split fat exceeded 80 per cent.

15. Much experimental work has been carried out in recent years on the absorption of fat, the most important being by FRAZER, " Fat Absorption and Metabolism " (1938); FRAZER and STEWART (1939). A review of the subject, " Fat Absorption and its Relation to Metabolism," by FRAZER (1940), has also appeared. In these papers, from which I have quoted freely in paragraphs 16 to 21 below, FRAZER has put forward the evidence in favour of the fat : fatty acid partition hypothesis in human fat absorption. His classification is as follows :—

LIPIDS

- FATS
  - GLYCERIDES (neutral fats)
  - FATTY ACIDS SOAPS
- PHOSPHOLIPINS (Phosphatides)
  - LECITHIN SPHINGOMYELIN (choline-containing)
  - KEPHALIN (non-choline containing)

CHOLESTEROL

- FREE CHOLESTEROL
- CHOLESTERYL ESTERS (esters of cholesterol and fatty acids)

For those others who, like myself, find a difficulty in memorising formulae, the following are set down :—

CH_2OH	$CH_2O.CO.R$	$CH_2O.CO.R$	$Ca(O.CO.R)_2$	$CH_2O.CO.R$
$CH\ OH$	$CHO.\ CO.R$	$CHO.\ CO.R'$	$Na\,O.CO.R'$	$CHO.\ CO.R'$
				$\quad OH$
CH_2OH	$CH_2O.CO.R$	$CH_2O.CO.R''$	$KO.\ CO.R''$	$CH_2O.P{=}O$
				$\quad O.CH_2.CH_2$
Glycerol	Triglyceride	Mixed glyceride	Soaps	Lecithin $\quad HO{-}N(CH_3)_3$

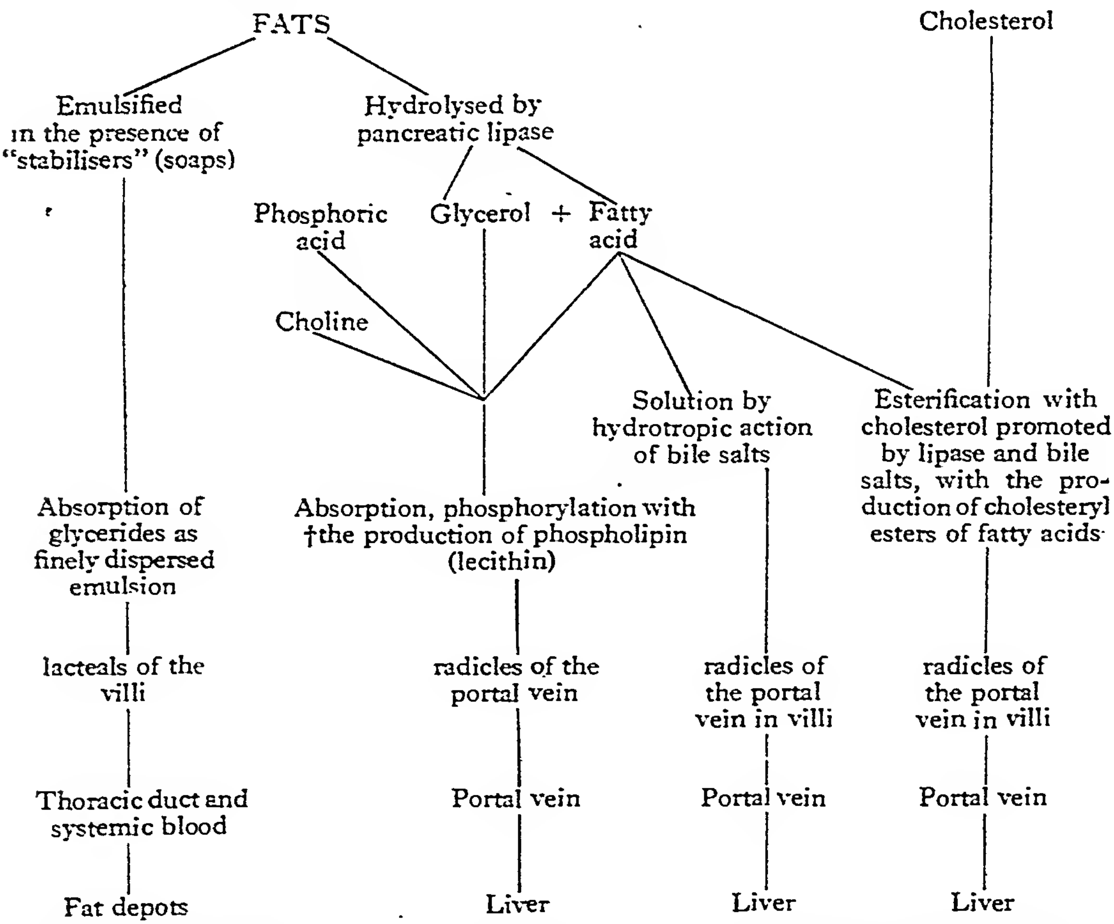
(R R′ R″ represent fatty acid radicals)

Lecithin is a phospholipin in which glycerol is united to two molecules of fatty acids, and to the base choline by phosphoric acid.

16. Glycerides are split in the small intestine by the pancreatic ferment lipase into glycerol and fatty acids. There is no evidence that the lipase present

22. Upon this hypothesis of "partition" as enunciated by FRAZER, I believe it is possible to build a theory which would explain the known facts concerning sprue. Normally the mode of absorption, the pathway to be followed, the destination and subsequent metabolism of fat is predetermined at the moment when lipolysis is taking place. Split fat follows one path, unsplit fat another. The unsplit fat (glyceride) fraction absorbed in the form of a finely dispersed emulsion passes by the lymphatic path to the fat depots while the fatty acid fraction passes *via* the portal vein to the liver partly as phospholipin, partly as cholesteryl esters, some possibly in solution effected by the hydrotropic action of bile salts. Some phospholipin may alternatively pass by the lymphatic route. ARTOM and PERETTI (1936) estimated that some 2 per cent. of the total fat in the thoracic duct was made up of phospholipid and fatty acid.

The accompanying diagram will serve to show the main points of this scheme at a glance.



A diagrammatic scheme according to the "Partition" theory showing the main paths followed by the two fractions: neutral (unsplit) fat and (split fat) fatty acid. The † the point of failure in sprue as herein suggested.

as suggested by VERZÁR, FRAZER says, "Under no circumstances can this evidence be regarded as demonstrating the essential nature of lipolysis" . . . "on the available evidence it is not possible to link phosphorylation with triglyceride synthesis." That resynthesis may occur in the intestinal mucosa is suggested by the fact that triglycerides may be recovered from the thoracic duct after feeding with monoglycerides or fatty acids, but according to FRAZER these experiments "show perhaps that a mechanism exists whereby resynthesis can occur but they do not demonstrate that it is either normal or essential."

18. In regard to the split-fat moiety, FRAZER states "There is, however, evidence that fatty acids may undergo phosphorylation, not as an intermediary in glyceride resynthesis but in the production of phospholipins." He and others using "tagged" fatty acids have shown that the cells of the intestinal mucosa are able to form phospholipin (lecithin). It was possible to trace the fatty acid from the ingested material to the formed lecithins. ARTOM and FREEMAN (1940) suggest that lecithin is the only type of phospholipin involved in the transport of fatty acids.

The destination of these phospholipins is the liver *via* the portal vein. Unsaturated fatty acids are preferentially selected for phospholipin synthesis but, with adequate supplies of fatty acid, relatively saturated and unsaturated fatty acids are selected alternately.

19. There is a close association between cholesterol and fat in the diet; the former is not absorbed unless fat is also present in a quantitative relationship. There is reason to believe that cholesteryl esters are formed in the intestinal cells. Lipase and bile salts are probably factors in promoting esterification with fatty acids in the intestine or in the cells of the intestinal mucosa; if this is the case some of the absorbed fatty acid will be taken up in this way and the cholesteryl esters thus formed may pass to the liver in the portal blood.

20. Depot fat, as has been mentioned above, is without doubt derived directly from absorbed neutral fat and mainly consists of triglycerides of low iodine value. When there is a surplus of fat in the food the depot fat resembles the ingested fat; when small in amount the depot fat may be more variable. Depot fat undergoes a "turnover," the half lifetime being a week. The amount of fat going to the depot will depend on the relationship of the quantity of fat in the diet to the lipolytic potentialities of the individual; obesity may be the expression of relative pancreatic inefficiency.

21. In regard to fat metabolism it is affected not only by quantity but by quality of the ingested fat.

The essential nature of certain unsaturated fatty acids and the importance of vitamin B_6 (pyridoxin) in their utilization form another link between the composition of the diet and the ultimate fate of absorbed fat.

Since choline enters into the composition of phospholipin, synthesized in the intestinal mucosa, the choline content of the diet may exert an essential function.

This I believe to be a more correct interpretation than that given by MILLER for coeliac disease when he states that "intestinal hurry" allows of little saponification while in the absence of hurry saponification is much greater. When HURST states that the excess of soaps in sprue causes irritation and diarrhoea he does not specify the soap. He states that sigmoidoscopic examination after a soap enema revealed congestion of the colon, but doubtless the soap used was not a calcium soap. The old "Batavia powder" containing chalk obtained from the powdered cuttlefish bone doubtless gained for its inventor a considerable reputation owing to its action in arresting the diarrhoea and replacing loose evacuations by massive solid stools.

In coeliac disease similar circumstances may be found. POYNTON and PATERSON (1914) remarked the non-diarrhoeic type of coeliac disease, and MILLER and PERKINS (1923), after pointing out, in that disease, the great preponderance of fatty acids and soaps over neutral fat, note that the stools may be well formed if the fatty acids are present chiefly as soaps.

Megacolon has often been given as a point of distinction between coeliac disease and sprue. Its onset in coeliac disease, as remarked by BENNETT (1934) has been noted to coincide with the cessation of diarrhoea, thus pointing, I believe, to calcium soap production as the cause of that condition. Megacolon is but rarely reported in sprue, but I think that routine barium enema examination in cases of sprue, especially those without diarrhoea, would demonstrate perhaps an unexpected proportion with dilatation and redundancy of the large bowel.

In the case recorded by HURST (1942b) the association of dilated and elongated pelvic colon with high soap content in the faeces (fatty acid 34, soap 22, neutral fat 7) is noteworthy, the process being rather analogous to what happens as the result of the delayed passage of fatty material in the small intestine. The loss of calcium to the body in sprue gives rise to tetany, in the child with coeliac disease to bony defects, another point commonly mentioned as distinguishing the two conditions. It seems possible, however, that this difference is merely one associated with age incidence. In the growing child calcium may well be more easily mobilized, metabolism a more "fluid" and unstable process, than in the adult. This might account for osteoporosis in coeliac disease and its absence in sprue.

I am inclined to think that SCOTT's theory (1923) of parathyroid deficiency does not play any part in calcium absorption though doubtless the parathyroid hormone is a factor in the regulation of calcium metabolism in the body.

26. The suggestion is made that "the physiological lesion" in sprue resulting in deficient absorption of fatty acids is a defect in phosphorylation. Loss of neutral fat and calcium are secondary.

27. Turning next to the question of carbohydrate absorption, as has been stated above, normal breakdown of starch by amylase and maltase takes place with the production of a series of dextrins and ultimately glucose. Cane sugar

23. In sprue it is suggested the primary defect is one of failure of a complex enzymatic reaction, probably phosphorylation, involved in the absorption of the fatty acid fraction only, the specific result being the non-absorption of fatty acids. (A defect in phosphorylation of glucose, I believe, also occurs as will be shown below.)

The loss of neutral fat such as occurs in sprue I suggest may be due to the mechanical effect of the mass of fatty acid in the bowel. This might interfere with emulsification; faulty soap formation might be a factor or lack of phospholipid.

The variability in the neutral fat loss in the faeces could, I think, thus be explained, as also the well-recognized fact that loss of depot fat is not necessarily marked. MILLER (1921) remarked this same point in coeliac disease.

In many cases of sprue, however, loss of depot fat is considerable, explained I think by the fact that there is a "turnover" of depot fat, *i.e.*, fat is constantly leaving the depots and if not replaced owing to interference with neutral fat absorption, wasting and loss of weight occurs. The loss of neutral fat is seldom more than a third, and when interference with normal absorption is abated weight is rapidly restored.

That lowered values for blood lipids occur in sprue has, of course, been shown by FAIRLEY and others and that restoration to normal follows treatment by liver extracts is well known. BARKER and RHOADS (1937), working on the post-absorption curve for blood lipids in sprue, concluded that liver extract exerts a specific effect in converting a mal-absorbing intestine to normal function.

24. Hypocalcaemia, so generally found in sprue, is again probably due to loss of calcium in the stools, "fixed" by excess of fatty acids with the formation of calcium soaps, rather than to any deficient power of absorption. As stated by FAIRLEY (1936), "Not all cases of steatorrhoea show hypocalcaemia but where hypocalcaemia is found the faecal fat (*i.e.*, fatty acid) is high, and invariably so when tetany is present," but he omitted to demonstrate an association between hypocalcaemia and high calcium-soap content in the stool. He goes on to say, "As a rule the response to calcium per os was excellent provided the patient simultaneously received a graded high protein diet, low in fat and carbohydrate and oral liver extract," a statement which to read true should be inverted—provided the amount of fatty acid in the bowel is diminished by treatment, loss of calcium will not be so great and calcium given by the mouth will be available to the body. This author notes in another paragraph that calcium administered by the mouth may not be satisfactory when steatorrhoea persists but improvement in blood calcium values may occur when the fat intake is cut down further.

25. An excess of ingested calcium, though in no way affecting the total amount of fat in the sprue stool, may often diminish the diarrhoea as calcium soaps tend to solidify the stools and lessen the irritative effect of the fatty acids on the large bowel.

This I believe to be a more correct interpretation than that given by MILLER for coeliac disease when he states that " intestinal hurry " allows of little saponification while in the absence of hurry saponification is much greater. When HURST states that the excess of soaps in sprue causes irritation and diarrhoea he does not specify the soap. He states that sigmoidoscopic examination after a soap enema revealed congestion of the colon, but doubtless the soap used was not a calcium soap. The old " Batavia powder " containing chalk obtained from the powdered cuttlefish bone doubtless gained for its inventor a considerable reputation owing to its action in arresting the diarrhoea and replacing loose evacuations by massive solid stools.

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27. Turning next to the question of carbohydrate absorption, as has been stated above, normal breakdown of starch by amylase and maltase takes place with the production of a series of dextrins and ultimately glucose. Cane sugar

(sucrose) is similarly hydrolysed by invertase to "invert sugar"—glucose and fructose (laevulose). To consider glucose first—the evidence for deficient absorption is founded on two observations, namely, carbohydrate fermentation in the bowel and a low, flat blood sugar tolerance curve. In regard to the first point BENNETT and HARDWICK believed that the gassy distension of the bowel was due to fermentation of starch, but this is not so, as pointed out by HURST. HURST, in turn, suggests that the intestinal distension is due to deficient gas absorption whereas it would seem obvious that the gas is produced by the fermentation of unabsorbed glucose.

The original view that held the flat oral glucose tolerance curve was due to deficient absorption was later disputed. SWEENEY (1927) stated "the ingestion of a high carbohydrate diet by normal individuals has been shown to result in a flattened oral glucose tolerance curve" or, in other words, an increased tolerance for carbohydrate. HIMSWORTH (1935) explained this finding as due to the subject being rendered highly sensitive to insulin. He has shown that when 50 grammes of glucose is given per os to a healthy subject on a fat diet, a blood sugar curve indistinguishable from that in diabetes is obtained; but when to a subject on a carbohydrate diet, a flat curve is seen, due to an increased removal of sugar from the blood. HIMSWORTH (1934) had suggested that the flat curve observed in sprue was comparable to that obtained in the subject on a carbohydrate diet, arguing that the sprue patient was for all intents absorbing a diet consisting of carbohydrate to the exclusion of fat. He considered that "we are justified in saying that a flat curve is not characteristic of sprue or coeliac disease." This explanation was widely accepted and incorporated in the views put forward by various writers, including HURST and MANSON-BAHR (1940), notwithstanding the fact that HIMSWORTH (discussion on FAIRLEY'S paper, 1936) had withdrawn his original suggestion. It is therefore again generally agreed that in sprue there is deficient absorption of glucose from the bowel. MANSON-BAHR'S statement that the low curve is not due to destruction in the bowel or impairment of absorption is, I believe, incorrect.

28. The failure to absorb glucose is not a contingent of diarrhoea as pointed out by BENNETT (1934) nor is the failure the result of steatorrhoea as is well shown in a case of gastro-jejuno-colic fistula published by FAIRLEY and KILNER (1931). In that case the stools contained a proportion of fat comparable with that found in sprue but sugar was normally absorbed and the glucose tolerance curve was normal. The case of regional ileitis given by TODD and others (1940) illustrates the same point. After five re-sections only 3 feet of small intestine remained, yet despite this fact and steatorrhoea causing a loss of calcium, 99 per cent. of the carbohydrate was absorbed.

Again, in congenital steatorrhoea due to pancreatic disease, in spite of the fact that the greater part of the ingested fat appears in the stools, in this case unsplit, a high normal sugar tolerance curve was demonstrated by HARPER (1938).

29. That the absorption of glucose is not a simple process of diffusion appears probable. VERZÁR believes it is absorbed from the small intestine with selective rapidity in experimental animals. The proportional rates of absorption by rats of various sugars have been assessed as follows: galactose 115, glucose 100, fructose 44, mannose 33, sorbose 30, xylose 30, arabinose 29, rhamnose 29. It is immediately to be noted that the two sugars which undergo rapid absorption are those which in their inter-reactions in the body are commonly phosphorylated. It would be reasonable to suppose, and there is evidence for believing, that phosphorylation occurs in absorption of glucose from the intestine, according to VERZÁR, in order to keep up a steep diffusion gradient.

Mannose and other sugars with low absorption rates are absorbed, it is believed, by simple diffusion; they are sugars which are not phosphorylated in the body.

30. Fructose (laevulose) appears to occupy an intermediate position. Its absorption appears to be unassociated with phosphorylation, yet it is sufficiently rapidly absorbed to place it in a different category from mannose and the other sugars.

Fructose can be utilized by the sprue patient just as by the diabetic, thus affording, for the first time I believe, an explanation of the old method of treatment by strawberries which, though perhaps it is not generally recognized, contain protein, practically no fat and carbohydrate as fructose. In this lies their value as a diet in sprue and not, I believe, as MANSON-BAHR has suggested, in their vitamin content.

In this connection it may be well to remember that the banana, which has also been used in sprue, contains not only protein and fructose, the former in twice the amount contained in strawberries, but also starch.

CASTELLANI (1937) found the laevulose test in sprue to be normal. The exact significance of that author's rhamose test is not clear.

31. It appears possible, therefore, that the failure in glucose absorption in sprue, as has been suggested in the case of fatty acid, is due to a failure in phosphorylation. It is I believe the principal, if not the only, process primarily at fault in sprue, but it is not governed, as believed by VERZÁR, by the adrenal.

The introduction into the molecule of the trivalent phosphoryl radicle $\equiv P:O$, known as phosphorylation, ensures a greatly increased combining power or potential reactivity. When one acid group is esterified, with glucose for instance, two acid groups remain free to unite with bases, with the amino groups of proteins, with alcohols, etc. This process is well exemplified in the formation of lecithin (*vide supra*).

Phosphorylation is effected, or catalysed, normally in the body by enzymes; the actual enzymes involved in the processes under immediate consideration have not been determined but there is some evidence for believing that some part of the vitamin B_2 complex is involved in this catalysis. Sprue might then

take its place alongside pellagra among the deficiency diseases, due to a fault in one of the complex enzyme systems of the body.

When FAIRLEY (1936) stated that in his cases the histories disclosed no evidence of dietetic deficiency, less was known upon this question than at the present time.

32. The idea that a deficiency was the primary cause of sprue arose as the result of the investigations of RHOADS and MILLER (1934) when studying the anaemia in sprue. They found, and the fact was emphasized by CASTLE, RHOADS, LAWSON and PAYNE (1935) that not only did the anaemia respond to liver extracts but that all the other symptoms of sprue did so at the same time. Extracts of liver given by mouth availed little, it was necessary to use crude extracts parenterally; whereas a single injection was sometimes followed by a cessation of diarrhoea in 24 hours, relapse occurred unless treatment was continued and in refractory cases as much as 20 c.c. daily might be necessary.

They believed that liver extract supplied some necessary vitamin. In view of their results they pointed out how difficult it is to eradicate the conception that diet is the main factor in the treatment of sprue—" diet is at best an uncertain method of obtaining liver extract for the internal economy of the organism." Nevertheless, the " liver soup " of two generations ago had played a useful part in treatment. The dietetic treatment by high protein, low carbohydrate and fat, which later formed a part of the general therapy of sprue, we should recognize as having no specific object beyond unloading the bowel of a mass of material which it cannot use and which secondarily causes the loss of other substances.

33. Treatment by means of liver extracts has, of course, persisted to this day though the quantities used have often been too small. It was demonstrated that adequate amounts of liver extract not only stopped the diarrhoea but restored glucose absorption and the glucose tolerance curve returned to normal.

With advancing knowledge concerning vitamin B_2 complex and its relation to the liver, a number of observers turned their attention in that direction, both in regard to sprue and idiopathic steatorrhoea. BRULL (1938) believed that non-tropical sprue was the expression of an avitaminosis. ANTOGNINI (1941) saw in steatorrhoea a B_2 hypovitaminosis due possibly to a variety of causes which hindered vitamin B_2 absorption from the intestine. VEDDER (1940) suggested an anterior pituitary dysfunction with consequent non-absorption of vitamin B_2 complex as the basis of sprue. CHILDS and DICK (1940), however, have suggested a deficiency of some specific but unknown pancreatic factor in non-tropical sprue.

34. Following upon the splitting up of the vitamin B_2 complex and the identification of many of its components and later their use in pellagra, essays

with these substances have been made in sprue and in idiopathic steatorrhoea in Denmark, Germany and France, in the East and in this country.

DE LANGEN (1940) found that nicotinic acid yielded varying results which on the whole were not as good as those obtained with parenteral crude liver extracts. MANSON-BAHR (1941) is, however, quite enthusiastic about his results with nicotinic acid. [His paper is entitled "Treatment of Sprue with Vitamin B_2," but he really refers to nicotinic acid not riboflavin which is properly designated B_2. This paper needs careful reading as the term vitamin B_2 is variously used to refer to vitamin B_2 complex, vitamin B_2 and nicotinic acid. The same confusion of terms occurs in a "Review on Sprue" by the same author (1941).] It should be noted that in his series of cases treated with nicotinic acid, the dose was small, in nineteen 150 mg. per diem, in four 300 mg., and that in the majority, certainly in nineteen out of the twenty-three, crude liver extract was given in the form of "campolon" 2 c.c. daily and in seven 3 mg. riboflavin daily. MANSON-BAHR sums up: "The hypothesis is advanced that the sprue syndrome is mainly due to non-absorption or destruction of vitamin B_2 (*sic*) in the small intestine. . . . It may, therefore, be assumed that the disease which we recognize in tropical medicine as sprue represents the fully developed picture of small intestine deficiency and is presumably due to previous damage to the intestinal mucosa."

In the Review MANSON-BAHR follows IZOD BENNETT's lead of "chronic jejuno-ileal insufficiency" and speaks of "chronic jejuno-ileal inefficiency" due to damage to the mucosa of the small intestine. He goes on to say "An analysis of these assembled facts, based mainly upon clinical observations, would indicate that what has already been prophesied by ELDERS (1917) and NICHOLS (1934) and others that sprue is the expression of disease of the small intestine is true, and it now appears to be due to an avitaminosis through faulty absorption."

Further, this author suggests that "sprue is the result of dysfunction of some specialized cells in the same manner as is now generally accepted for pernicious anaemia" and produces a diagram to show the sites of these several groups of specialized cells associated specifically with pernicious anaemia, sprue and pellagra.

35. If I understand MANSON-BAHR correctly, put in so many words, he suggests that as a result of previous intestinal disease, damage is done to certain cells of the jejuno-ileal mucosa whose function is bound up with the absorption of nicotinic acid and that sprue results from the deficiency of nicotinic acid.

The first part of his argument is based doubtless upon figures published by MANSON-BAHR and WILLOUGHBY (1930) when it was shown that 40 per cent. of his 200 sprue patients gave a history of preceding intestinal disorder. This argument loses weight if it be recognized that in 60 per cent. there was no such history and if at the same time a second figure is taken into consideration,

viz., 30 per cent. had suffered previous malaria; these facts may mean no more than that the stores of various vitamins had been depleted by the illnesses as they are known to be by any infective or febrile condition and by the associated restricted diet, just as happens with Castle's factor in pernicious anaemia.

Again it seems rather anomalous that the sprue patient who, according to MANSON-BAHR, has lost the power to absorb nicotinic acid, should respond satisfactorily when treated with small doses of that substance by mouth.

Further, there is no evidence, as far as I am aware, that certain cells have any specific function in regard to the absorption of nicotinic acid. Lastly, if sprue be a nicotinic acid deficiency it seems strange that pellagra is not a common concomitant. That pellagra and sprue may occur in the same patient is well known, but with the view, held by some writers, that the skin condition commonly seen in sprue or the pigmentation in coeliac disease is pellagrous, I am inclined to disagree. I must conclude that Manson-Bahr's hypothesis will not meet the facts, it also offers no explanation of the pathogenesis of the condition we call sprue.

36. We must return, therefore, to the one piece of evidence, the one fact in regard to treatment, beyond all doubt—the response to the parenteral administration of large doses of crude liver extracts as originally demonstrated by RHOADS and MILLER (1934). As stated by LEPORE (1941) treatment with liver extracts is followed by restoration of normal bowel function and normal absorption.

According to the view put forward in this article, liver extracts contain the factor (or factors) or some essential component, which operates or catalyses the phosphorylation of glucose and fatty acids. This factor or its component would almost certainly be a member, known or unknown, of the vitamin B_2 complex.

37. To consider glucose first. The first step in the capture of the glucose molecule by any living cell appears to be by its combination with phosphoric acid to form a monophosphoric acid ester; esterification (or the process of union of an alcohol with an acid) occurs easily with a weak acid like phosphoric acid which also readily esterifies with glycerol and other alcohols, with the hydroxyl groups of fatty acids, etc.

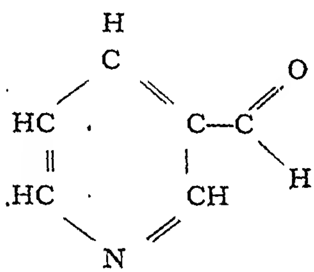
The process is speeded up by enzymes—esterifying catalysts or phosphatases; the process may be reversible, *i.e.*, splitting of the ester by hydrolysis may occur, sometimes by the same catalyst.

Now diphosphopyridine nucleotide (which is also known as coenzyme I and consists of a molecule each of adenine and nicotinic acid amide united to a molecule of pentose in turn connected by two molecules of phosphoric acid), in addition to its action as a hydrogen " carrier " or " acceptor " in dehydrogenase systems, may also function in the transfer of phosphoric acid, as it can supply material for the formation of adenylic pyrophosphate. The mucosa of the small bowel has a high content of phosphatase.

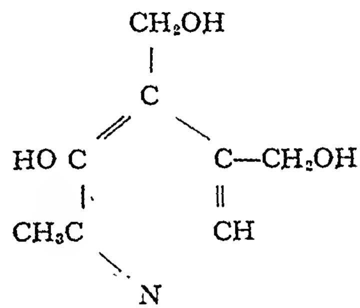
There is therefore some reason for believing that nicotinic acid may be implicated in the phosphorylation of glucose on absorption from the intestine. The same is true in regard to glycerol in its absorption from the bowel. Diphosphopyridine nucleotide is, of course, the coenzyme (agon) or active component of the enzyme and is united to the apo-enzyme (pheron), a protein, and it is possible that coenzyme I may be united to different protein apo-enzymes in different reactions.

Riboflavin has also been stated to be a phosphate "carrier," so that while nothing is known with certainty concerning the enzymes catalysing phosphorylation of glucose, glycerol, fatty acids on absorption from the bowel, it is probable that coenzyme I or some analogous substance does play a part.

38. The structurally closely allied vitamin B_6 or pyridoxin may also be implicated. It is the factor which in the past was variously called filtrate factor I of Lepkovsky, Jukes and Krause, the "H" of Booher and of Hogan and Richardson, the "Y" factor of Chick and Copping, and is known as "rat anti-acrodermatitis factor"; by constitution 4, 5, dihydroxymethyl, 2 methyl, 3 hydroxypyridine.



nicotinic acid



pyridoxin

Whether pyridoxin plays any important part in human metabolism is not known. Most of the work concerning this factor has been carried out on rats by a number of investigators, including Burr, Burr and Miller (1932), Birch and György (1936), Hogan and Richardson (1936, 1941), Turpeinen (1938), Halliday and Evans (1937), Quackenbush, Platz and Steenbock (1938, 1939), Hume *et al.* (1938), Nunn and Smedley-Maclean (1938), Birch (1938), Salmon (1941), Lepkovsky *et al.* (1942), etc.

Birch (1938) concluded that there were two factors concerned in the cure of rat acrodynia—(*a*) a water-soluble factor present in yeast and wheat germ which is pyridoxin or vitamin B_6; (*b*) a factor present in the fatty acid fraction of certain oils and fats similar to linoleic acid—possibly arachidonic acid. Vitamin B_6 is in some way connected with the metabolism of unsaturated fatty acids. The amount of vitamin B_6 ingested by a rat fed a fat deficient diet appeared to determine the kind of dermatitis which it will develop. Birch

says, " whether pyridoxin is concerned in the oxidation or in mobilization and transport of fatty acid is not yet known. The possibility also remains that vitamin B_6 merely combines with the unsaturated fatty acid to form some essential constituent of the cell similar to lecithin or its allied substances. This latter possibility seems likely as vitamin B_6 appears to have similar properties to choline." On the other hand, it has been suggested that part of the pyridoxin may be combined with protein as the prosthetic group in the same way as nicotinic acid.

RICHARDSON and HOGAN (1941) consider that there are three factors involved in the cure of acrodynia in rats : (*a*) pyridoxin, (*b*) linoleic acid, and (*c*) pantothenic acid (Factor II, Bios, rat growth factor or chick dermatitis factor), but FOY and CERECEDO (1941) have shown that the specific type of dermatitis in rat acrodynia is due to vitamin B_6 deficiency while a non-specific dermatitis is due to pantothenic acid deficiency. LEPKOVSKY (1942) puts his view in rather a different way, saying that since acrodermatitis in rats is the result of a pantothenic acid deficiency superimposed upon a pyridoxin deficiency, it is uncertain whether dermatitis is a reliable expression of pyridoxin deficiency. BIRCH had suggested that possibly " In the absence of an adequate supply of vitamin B_6 the animal is unable to make proper use of the unsaturated fatty acids ; or, alternatively, in the absence of adequate amounts of unsaturated fatty acids, the animal is unable to utilize its vitamin B_6."

The following figures, given by JUKES (1941) show the pyridoxin values for various substances :—

Corn oil, 20,000 ; wheatgerm oil, 15,000 ; peanut oil, 5,000 ; linseed oil, lard, egg yolk, 2,500 ; peanut, 1,660 ; wheat germ, soya, 1,250 ; flaxseed, 1,000 ; dried pork liver, 500 ; oatmeal, 330 ; cheese, 250 ; dry beef muscle, 125 ; milk, 40 ; vegetables and fruits, 10 to 60.

39. The facts briefly surveyed above concern pyridoxin and its relation to unsaturated fatty acid metabolism within the body of the rat. In a general way many of the processes undergone by food substances during digestion and absorption from the intestine appear to be analogous to those that occur in their metabolism after absorption and it seems possible, in view of BIRCH'S suggestions, that pyridoxin may play a part in the absorption of unsaturated fatty acids by the intestinal mucosa and their incorporation into phospholipids and therefore be a factor in the pathogeny of sprue. It seems possible that the geographical distribution of sprue might be bound up with the nature of the unsaturated fatty acids in the diet. The number of fatty acids contained in any single article of diet may be considerable and the variation in quantity of each marked. To give a single example—fatty acids in dairy butter (CROWTHER and HYND in England and LEBBERT in the United States) in per cent. of total acids :—

Fatty Acid.	*England.*	*United States.*
Butyric	4·45	3·2
Caproic	1·45	1·8
Caprylic	1·00	0·8
Capric	1·10	1·4
Lauric	3·55	3·8
Myristic	20·13	8·3
Palmitic	15·24	24·4
Stearic	1·08	13·9
Dihydroxystearic	0·68	4·0 (linoleic)
Oleic	45·47	38·4

40. Little is yet known about pyridoxin deficiency in man. SPIES, BEAN and ASHE (1939) found that certain symptoms in pellagra—nervousness, insomnia, irritability, abdominal pain, weakness and difficulty in walking—resistant to other forms of treatment, responded to pyridoxin. SMITH and MARTIN (1940) have reported rapid healing of cheilosis in two cases of pellagra and one of coeliac disease but only partial response in a complicated case of sprue even when riboflavin and nicotinic acid were added.

What these observations mean it is not possible to say ; they may suggest interference with phospholipid metabolism or absorption. Similarly, the skin changes in sprue and other steatorrhoeas (and those in pellagra even) may have a basis in faulty phospholipid metabolism. HANSEN (1932-3) demonstrated that the serum fatty acids are less unsaturated in infantile eczema than in normal children, and BURR and BROWN (1932-3) found the skin lipids in rat dermatitis were abnormal.

ANTOPOL and UNNA (1939) found in the various layers of the skin of vitamin B_6 deficient rats hyperkeratosis, intracellular oedema, acanthosis and atrophy of hair and sebaceous follicles, changes which responded to treatment with the vitamin. In this connexion it may be remembered that, as shown by KOPPENHOEFER (1938), normally different lipids occur in the different layers of the skin—near the subcutaneous region triglycerides, in the corium phospholipids and sterols, in the epidermic region phospholipids, sterols and waxes.

41. There are other points in connexion with phospholipins which may have a bearing on sprue, including the anaemia of sprue, a symptom which has never received any explanation.

In pernicious anaemia, as in some other types of anaemia, there are found low plasma values for phospholipins and cholesterol esters, but an increased neutral fat. Normal values are re-established by treatment with liver extracts as shown by WILLIAMS *et al.* (1937) and KIRK (1938); previous observers include MACPHERSON (1917) and MULLER (1930). It appears very possible that haemopoiesis is bound up in some way with normal absorption and metabolism of phospholipids probably containing highly unsaturated fatty acids. The relationship of the macrocytic anaemia of pernicious anaemia and sprue and other macrocytic anaemias might thus be explained. Pyridoxin appears to be in some way related to haemopoiesis. CHICK and COPPING (1930) found

evidence for believing that vitamin B_6 has some connexion with haemoglobin formation.

FOUTS *et al.* (1938, 1939) showed that puppies and dogs fed a diet deficient in vitamin B_6 developed a microcytic anaemia. This was confirmed by MCKIBBIN *et al.* (1942) in pyridoxin deficiency in dogs, but STRUTT *et al.* (1941) had failed to cure the anaemia with vitamin B_6 and believed some other factor was involved.

In man there are but few records. SMITH and MARTIN (1940) report the clearing up of the anaemia in a single case of pellagra treated with pyridoxin. VILTER, SHIRO and SPIES (1940) treated three cases of pellagra with macrocytic anaemia and two of pernicious anaemia by intravenous injections of crystalline vitamin B_6, 50 to 100 mg. each day for 10 days. In 48 hours there was an increased sense of well-being and on the 5th to 8th days there was a slight but definite reticulocytosis but it was obvious that pyridoxin did not act as the specific antianaemic factor.

KARK *et al.* (1940) administered the same preparation to six patients suffering from various deficiency states—two endemic pellagra, two alcoholic pellagra, one idiopathic hyperchromic anaemia, one nutritional macrocytic anaemia. Pyridoxin was given for from 6 to 21 days without improvement.

These trial treatments with pyridoxin, it may be pointed out, were carried out empirically without reference to unsaturated fatty acid and phospholipid. While nothing has been proved, there is much regarding pyridoxin and unsaturated fatty acids which suggests a possible association with sprue.

SUMMARY.

42. *A.* Criticisms are offered upon theories previously enunciated concerning the causation of sprue and upon some of the observations upon which they are founded.

B. An attempt is made to suggest the lines along which a solution of the problem may lie.

C. Evidence is adduced in favour of a theory based upon the "partition" hypothesis which predicates for unsplit (neutral) fat and for fatty acids a different mode of absorption from the intestine, a different route after absorption, a different composition during transport, a different destination and a different rôle in the bodily metabolism.

D. The theory now tentatively put forward regarding the pathogeny of sprue enlists the following considerations :—

(1) The deficient absorption of fat is limited primarily to loss of power to absorb the fatty acid moiety and cholesterol.

(2) There is no loss of power to absorb neutral fat (glyceride) but there is a deficiency of neutral fat absorption due, secondarily, to non-absorption of fatty acid.

(3) There is also a primary loss of power to absorb glucose which results in a low flat oral blood sugar tolerance curve, glucose no longer being selectively absorbed by an active process but only by diffusion.

(4) The same is true of glycerol formed by the splitting of neutral fat.

(5) The loss of power to absorb fatty acid, glycerol and glucose is due probably to failure of phosphorylation. They are all substances, as opposed to many others, which require to be phosphorylated on absorption by the intestinal mucosa.

(6) Neutral fat is normally absorbed as a finely dispersed emulsion and does not require to be phosphorylated. Failure of absorption is secondary to non-absorption of fatty acid.

(7) Fructose (laevulose) is normally absorbed without phosphorylation and utilized by the sprue patient as by the normal subject.

(8) Loss of calcium to the body is due to " fixation " by fatty acid in the bowel with the formation of insoluble soaps.

(9) Loss of phosphorus is due to defect in phospholipid formation resulting from failure of phosphorylation.

(10) The effect of the mass of unabsorbed fatty acid and collection of gas due to the fermentation of unabsorbed glucose upon the small bowel is to produce intestinal delay in the small bowel and a passive distension, together with the other signs commonly demonstrated by radiographic examination.

(11) The primary failure in sprue, as above stated, is one of phosphorylation, not due, as suggested by VERZÁR, to lack of adrenal hormonal control, but the result of defective enzymic action.

(12) The enzyme or enzymes which catalyse phosphorylation — the " carriers " of phosphoric acid—probably have as the active part of the molecule coenzymes embodying some member or members (identified or unidentified) of the vitamin B_2 complex—all present in crude liver extracts. These may include riboflavin, nicotinic acid and pyridoxin. It may be remembered that choline is sometimes now included in the B_2 complex.

(13) Whether the same enzyme catalyses the phosphorylation of all of the several substances is uncertain.

(14) Since normally the phospholipids (lecithins) synthesized by the intestinal mucosa from fatty acid, glycerol, phosphoric acid and choline contain unsaturated fatty acids it is possible that desaturation occurs at the same time as phospholipid synthesis. In view of the close association of pyridoxin with unsaturated fatty acids, it seems possible it may here be at fault in sprue.

(15) The nature of the fatty acids in the diet may be a factor in determining the geographical distribution of the disease.

(16) The loss to the body of certain phospholipids containing highly unsaturated fatty acids may be a factor in the causation of the anaemia in sprue and of other nutritional anaemias.

(17) There are no pathological changes in sprue.

There is no secretory failure and no failure of absorption except that resulting from defect in phosphorylation.

(18) Sprue is properly placed among the diseases of malnutrition.

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THE MORPHOLOGY OF THE BLOOD IN DIMORPHIC ANAEMIA.

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DEFINITION.

Dimorphic anaemia is an anaemia which is due to two deficiencies, iron-deficiency and that of nutritional macrocytic anaemia. It is thus iron-deficiency anaemia complicated by nutritional macrocytic anaemia or may equally well be regarded as nutritional macrocytic anaemia complicated by iron-deficiency. The only justification for the new term of dimorphic anaemia is that it is a short descriptive title for a condition which may well be one of the commonest anaemias of the tropics and of other regions. Whether the creation of this new aspect of anaemia is justified will depend on whether it affords a clearer insight into the aetiology and mechanism of anaemia and replaces to a certain extent and in certain cases numerous other terms like hookworm anaemia, bilharzial anaemia, and so on.

MODERN TRENDS IN HAEMATOLOGY.

There are few aspects of medicine which have undergone more changes in the past 20 years than that of haematology. Some 20 years ago anaemia was classified into primary anaemia, which comprised pernicious anaemia and chlorosis; and secondary anaemia which was secondary to tuberculosis, nephritis, gastric ulcer, haemorrhoids, fibroids and other known diseases. This classification was rendered completely obsolete by the discovery that liver cured pernicious anaemia, in other words that it was a deficiency anaemia. Further work demonstrated that chlorosis and some of the secondary anaemias were due to iron-deficiency, and were cured by iron. At the same time further studies on cell morphology and cell size, revealed that pernicious anaemia and certain other anaemias which also reacted to liver had large cells in the peripheral blood, and the bone marrow showed certain changes which were characteristic of this group. To define this group better the large cells were called macrocytes, their diameters were measured by the Price-Jones curve and their cell volumes by the haematocrit, and the bone marrow was called megaloblastic. A host of new terms emerged which at first sight appeared redundant but which proved acceptable because of the greater insight they afforded into the mechanism of this group of anaemia. Similarly, iron-deficiency anaemia was found to be microcytic and to have a distinctive bone marrow picture. These two great

deficiency anaemias appeared usually as distinct clinical entities and were seldom associated in the same patient. Further, they stood at opposite poles of every modern haematological conception, one was macrocytic, the other was microcytic, and normal blood lay between the two, one was hyperchromic as regards the colour index and the other was hypochromic and normal blood lay in between. True, not every anaemia was found to be due to a deficiency, some were haemolytic, others had a more obscure origin, nevertheless the achievement of modern haematology consisted in the demonstration that by far the large majority of cases of anaemia seen in temperate regions were due to a single deficiency and were curable by correcting this deficiency. Amidst a plethora of new words this single fact emerged.

This idea has affected but little the classification of anaemia in the tropics. Here anaemia is regarded as occasionally primary in origin, usually it is regarded as secondary to known tropical diseases such as ankylostomiasis, bilharziasis, dysentery, malaria and others. Faced with a case of anaemia in the tropics the average clinician asks what other disease, usually what other tropical disease, is present; he does not ask what deficiency is present. This largely resolves itself into a search for malaria parasites, dysentery, hookworms, bilharzia, and other helminthic and parasitic diseases. Given modern methods of examining blood slides and stools it is certain that the large majority of anaemic patients are found to harbour some tropical helminthic or parasitic disease. Once this is found it is usually considered that the investigations may be concluded, for since it is believed that anaemia is a secondary disease, investigation is limited to detecting the causative disease. Occasionally it may cause some concern that a severely anaemic case has only scanty malarial parasites in the blood smear so that the case may not be one of just "malarial anaemia," or the hookworms may be very few and may not explain the presence of so severe a "hookworm anaemia." For the modern dilemma is that improved methods of detecting malaria parasites and hookworms demonstrate that in some parts of the tropics almost every one has one or other of these infections, even if in a very mild form. Can anaemia, however, be ascribed completely or only partially to the presence of these diseases? If, say, at autopsy on a case of anaemia, dying with a haemoglobin of 10 per cent., one hookworm is found, does this constitute a claim to classify it as a case of "hookworm anaemia"? If this solitary hookworm can hardly be regarded as a cause of death, are two hookworms an adequate cause, or three, or thirty, or three hundred or three thousand? Where is the dividing line? A clear answer can never come from this direction. Yet modern methods of examining stools claim that they can detect cases harbouring one gravid female hookworm.

The clinician may feel that in making the diagnosis of, say, hookworm anaemia or malarial anaemia he should be guided by papers purporting to describe the variety of anaemia which is under discussion. A careful perusal of papers issued on many tropical anaemias will leave him with the impression

that papers on, say, " malarial anaemia," have been compiled by persons very skilled in malaria who have collected as many cases as possible of anaemia with malarial parasites in the blood smear. Here, again, the finding of one parasite would constitute a claim to be admitted to a series purporting to describe malarial anaemia.

It appears quite certain that the tendency of modern haematology to regard anaemia where possible as a deficiency disease will have to overcome many difficulties in the tropics. For, to begin with, the main deficiency anaemia of the tropics, nutritional macrocytic anaemia (tropical macrocytic anaemia), is regarded as almost restricted to pregnant women, largely Indian pregnant women, and so is not considered in the majority of cases. There is much recent evidence to suggest that this impression is quite fallacious and it is difficult to understand why only pregnant Indian women should suffer from a deficiency of extrinsic factor in their diet. Of the other deficiency anaemias, pernicious anaemia is generally conceded to be very rare except in the white race, and iron-deficiency anaemia by itself and uncomplicated by hookworms is regarded as uncommon in the tropics. Deficiency anaemia is therefore regarded as uncommon in the tropics. A further difficulty to be encountered is that when an attempt is made to define the deficiencies present in cases of anaemia in the tropics it will be found that the problem is not a simple one, and that the deficiencies are usually multiple. This will lead many to abandon the notion of deficiency anaemia and they will return to the conception of secondary anaemia. A final difficulty will be encountered in the dissemination of the results obtained by those who investigate anaemia from the modern standpoint for they will describe largely iron-deficiency anaemia, nutritional macrocytic anaemia and a mixture of these (dimorphic anaemia). Those interested in, say, hookworm anaemia will not take cognizance of papers which describe nutritional macrocytic anaemia complicated by hookworms, for this would appear to introduce unwarranted complications. They will continue to scrutinize papers purporting to describe only " hookworm anaemia."

The recognition of dimorphic anaemia.

The dual deficiency present in dimorphic anaemia was clearly recognized by NAPIER and his associates (NAPIER *et al.* 1937, 1938 a and b) among pregnant Indian women. They found a confused blood picture in fifty-two cases of pregnancy anaemia and although unable to record cell volume (M.C.V.) or cell haemoglobin concentration (M.C.H.C.) they clearly demonstrated that the large majority of their cases suffered from a dual deficiency of iron and of the liver principle. HARE (1939, 1940 a and b) extended still further the work of NAPIER in pregnant Indian women, and elsewhere I have tried to appraise the significance of his contribution.

Most cases of severe anaemia seen in Africans in Uganda show clear evidence of a dual deficiency—that of iron and that found in nutritional macrocytic anaemia.

The deficiency is accentuated in the majority of cases by hookworms which increase any iron deficiency and of chronic malaria which demands an abnormal rate of blood production to compensate for the blood destruction, and therefore increased supplies of the raw materials of erythropoiesis. Previous to the elaboration of the idea of dimorphic anaemia cases had been classified as malarial anaemia, hookworm anaemia, and nutritional macrocytic anaemia as well as various mixed groups but great difficulties had arisen in this classification. Thus out of 318 cases classified in this way, 51 were considered to be due to malaria; but in this group the colour index varied from 0·4 to 1·3, the Price-Jones curve was often macrocytic and only half the cases responded well to large doses of quinine and iron. Similarly, in 119 cases of anaemia ascribed to hookworms the colour index varied from 0·4 to 1·3, the Price-Jones curve usually revealed some macrocytosis, and improvement, although often good, was sometimes very slight after giving much iron and administering repeated anthelmintics whereas on the other hand liver injections sometimes gave a reticulocyte response even in cases of hookworm anaemia which had a low colour index. This led to the abandoning of the usual classification in favour of the following :—

1. Ascertain if deficiencies occur and define them.
2. Ascertain what diseases are accentuating or causing these deficiencies or in any way destroying blood (*e.g.*, hookworm disease, malaria).
3. Ascertain what other infections or serious pathological conditions (*e.g.*, tuberculosis, nephritis) are present which may inhibit erythropoiesis.

Since it was presumed from the foregoing preliminary observations that iron-deficiency and nutritional macrocytic anaemia were often present, it was found difficult to define the extent of these two deficiencies. The following methods were employed :—

1. Determination of the colour index.
2. Estimation of the mean diameter, and plotting the Price-Jones curve.
3. Estimation of mean cell volume.
4. Estimation of mean cell haemoglobin concentration.
5. Assessing the response to various therapeutic agents of iron and liver.
6. Morphology of the peripheral blood smear.
7. Morphology of the bone marrow.

It was found that the first method, that is the determination of the colour index, had only a very limited value. Although in all cases having a low colour index it was considered that iron deficiency was severe, and that of nutritional macrocytic anaemia was slight or absent, and similarly if the colour index was high it could be presumed that the latter deficiency was severe, but little could be said about the absence of all iron deficiency. In addition many cases had a colour index somewhere within the normal range (0·85–1·15) and it was some time before it was realized that this was frequently due to a mixture

of the hypochromia and hyperchromia of the two deficiencies. In a similar way an estimation of the mean diameter revealed that most cases had a normal mean diameter. In passing it may be remarked that although halometric methods were employed in some hundreds of cases subsequent plotting of the Price-Jones curve revealed that halometric methods were so grossly inaccurate as to be positively misleading. The direct measurement of 50 or 100 cells by a micrometer eyepiece, although falling short of the precision of the Price-Jones technique, could be accomplished in some 10 minutes and gave reliable information. Even the full Price-Jones curve gave very unsatisfactory results, a matter which has puzzled several observers. (See the discussion after HAMILTON FAIRLEY *et al.*, 1938.) The estimation of M.D. in nutritional macrocytic anaemia has in the hands of all observers proved an unreliable method of estimating macrocytosis, since in many cases the M.D. lies within the normal range. This is due to the fact that the red cell in this anaemia is not so much increased in its diameter as in its thickness, mean cell thickness and mean cell volume being always increased (HAMILTON FAIRLEY *et al.*, 1938). It follows from this that if nutritional macrocytic anaemia (having a high or a normal M.D.) is mixed with the microcytic hypochromic anaemia of iron-deficiency (having a low M.D.) that the mean diameter of the resultant anaemia may vary from microcytic to macrocytic, but that the majority will be normocytic. Estimation of M.D. (mean diameter) will not detect dimorphic anaemia.

The Price-Jones curve, although usually suggestive of nutritional macrocytic anaemia and of dimorphic anaemia is often unreliable in the latter anaemia largely because, as discussed later, there is a very unequal spreading of the cells in the more anaemic cases, microcytes tending to accumulate in the central portions of the smear and macrocytes at the edges and at the tail. The Price-Jones technique assumes that cells are distributed evenly, yet the survey of many hundreds of blood smears from head to tail and the employment of a micrometer eyepiece has convinced me that the unequal spreading of cells in dimorphic anaemia renders fair sampling difficult. The usual Price-Jones technique is performed in the dark, no attempt is made to sample fairly different parts of the blood smear, an effort is made to obtain fields where the red cells are fairly widely separated, this occurs towards the tail of the smear and here the macrocytes predominate. For all these reasons the estimation of mean diameter and of the Price-Jones curve is misleading. In addition no one could ever expect a Price-Jones curve in every clinical case: simpler methods of examination must be found.

The estimation of mean cell volume, although it demands an electrical centrifuge, has proved a method of great value. From the centrifuged deposit the volume of the packed cells can be read and from this the mean cell volume (M.C.V.) and the mean cell haemoglobin concentration (M.C.H.C.) can be calculated if the red cell count is known and the haemoglobin can be calculated in grammes. Although each haemoglobinometer should be calibrated separately so that its scale stated as a percentage figure can be converted into grammes

of haemoglobin, yet most modern instruments are sold with a scale which can be converted into grammes of Hb. with a fair degree of accuracy. These two figures have proved of inestimable value in the detection of the two deficiencies. In this way some 174 cases have been classified as follows :—

31 macrocytic orthochromic anaemia.	11 normocytic orthochromic anaemia.
80 macrocytic hypochromic anaemia.	8 microcytic hypochromic anaemia.
43 normocytic hypochromic anaemia.	1 microcytic orthochromic anaemia.

It must be clearly understood what these terms mean. The normal range of M.C.V. given by PRICE-JONES, VAUGHAN and GODDARD (1935) was calculated on the basis of the normal range being plus or minus three times the standard deviation and was found to vary from 75·144 to 96·096 c.μ. These figures define microcytic, normocytic and macrocytic, and these terms (as used subsequently in this article) have no reference to M.D. or the Price-Jones curve. Similarly, these investigators found a normal range of mean corpuscular haemoglobin concentration (M.C.H.C.) to be from 28·17 to 34·35 per cent., and since it is unknown in haematology for M.C.H.C. to be abnormally high, these figures define hypochromia and orthochromia (as used subsequently in this article), and these terms have no reference to the colour index, in which hyperchromia can occur. The latter term means that the red cell (as in macrocytic anaemia) has more haemoglobin than the normal cell, whereas M.C.H.C. indicates the degree of saturation of the red cell with haemoglobin. The normal red cell is fully saturated so also is the cell in nutritional macrocytic anaemia and in pernicious anaemia. Hyperchromia as related to M.C.H.C. cannot and does not occur. The varying ways in which these terms of macrocytosis, microcytosis, hypochromia and hyperchromia are used has caused much confusion.

The question then emerged : what was the significance of these groups ? It was assumed that macrocytic orthochromic anaemia was uncomplicated nutritional macrocytic anaemia and that no iron deficiency was present. As discussed in my previous article test meals excluded the question of pernicious anaemia. It was also mentioned in that article that microcytic hypochromic anaemia and microcytic orthochromic anaemia were probably due to pure iron-deficiency. Lately, however, I have observed two cases of microcytic hypochromic anaemia who have had, first, a marked reticulocyte response to iron and then, when this had subsided, they were given half a pound of cooked liver daily and both showed a second reticulocyte response. They were, therefore, cases of dimorphic anaemia. Unless other cases are observed, however, it is not desired to stress this point, but it is theoretically possible, where iron-deficiency is very severe and the deficiency of nutritional macrocytic anaemia is but slight, that the former might dominate the dimensions of the red cell and microcytic hypochromic anaemia might result. By turning the tables it is theoretically possible that even macrocytic orthochromic anaemia might contain a masked iron deficiency.

The fact which came, however, to dominate the situation was the existence of two large intermediate groups of eighty cases of macrocytic hypochromic anaemia and of forty-three cases of normocytic hypochromic anaemia. What was the aetiology of these two groups ? In the first place all cases having serious intercurrent disease (nephritis, tuberculosis, hepatitis, etc.) were excluded since treatment was unlikely to succeed and it was unlikely that they were cases of deficiency anaemia. In this way some twenty-three cases were excluded leaving some sixty-two cases of macrocytic hypochromic anaemia and some thirty-eight cases of normocytic hypochromic anaemia in which anaemia appeared to be the primary condition. As pointed out in a previous communication, there appeared to be clear evidence that all the cases of macrocytic hypochromic anaemia were suffering from dimorphic anaemia and that the majority, although not all, of the cases of normocytic hypochromic anaemia had also this dual deficiency ; some of the latter group were, however, cases of pure iron deficiency.

The exact limits of dimorphic anaemia therefore cannot be strictly equated with cell volume and cell haemoglobin concentration but may comprise the following :—

1. Macrocytic hypochromic anaemia.
2. Normocytic hypochromic anaemia—occasionally.
3. Microcytic hypochromic anaemia—very occasionally.

The question of whether macrocytic orthochromic anaemia contains a masked iron deficiency, although suggested by RUSSELL (1941), has on the whole been inadequately investigated. If this is proved then the boundaries of dimorphic anaemia have been considerably enlarged. Until these matters have been decided the only satisfactory proof of the presence of dimorphic anaemia appears to be the double reticulocyte response to iron and to liver, the second deficiency being corrected when the first reticulocyte response has subsided.

Certainly no one like myself who has seen some dozen or more autopsies on cases heavily infected with hookworms and has seen blood clots in the intestine can doubt but that hookworms intensify the anaemia and that anthelmintics should be given at the earliest safe moment and repeated, if that again is safe, until all the worms have been expelled. One thing, however, has been demonstrated far too often to be doubted in the 119 cases, previously mentioned, of hookworm anaemia and that is that the reduction of the worm load and the administration of iron will in many cases not cure the anaemia and it will not reduce that element in the anaemia which is macrocytic and amenable to liver. It might be assumed that hookworms might cause some interference with the formation of intrinsic factor or might impede its interaction with extrinsic factor or the absorption, storage or utilization of the final liver-principle. If this were so, then expelling the worms should, as in infections by *Diphyllobothrium latum* (fish tapeworm), cure the macrocytic anaemia. This does not occur in my experience in hookworm anaemia.

In dimorphic anaemia, if a malarial infection is at all severe and is thereby still further increasing the degree of anaemia and inhibiting, as malaria does, erythropoiesis, no response can be obtained to correcting one or both deficiencies unless quinine, in the usual dosage for malaria, is given first for about 5 days. The response to quinine by itself is, however, often inadequate and only when iron and liver are subsequently or simultaneously given is the response adequate. In chronic malarial haemolysis iron is largely conserved inside the body, nothing is known concerning the fate of the liver principle. In chronic malaria dimorphic anaemia tends to show more liver principle deficiency than iron deficiency unless the hookworm load is heavy. Cases therefore approximate more to that of uncomplicated nutritional macrocytic anaemia, and often reveal but few parasites in the blood smear. At the same time the spleen may increase to a gross size and slight jaundice may occur. It has been the traditional position to ascribe these changes to chronic malaria, although better fed members of the community seldom show them and quinine and iron do not decrease appreciably either the size of the spleen, the slight but definite jaundice as estimated by a quantitative van den Bergh, or the degree of anaemia. Liver in adequate amounts cures the anaemia, cures the jaundice and will in early cases cause the splenomegaly to decrease considerably. In chronic splenomegaly, however, the spleen will not decrease in size. It is probable that many of the signs ascribed to chronic malaria are those of nutritional macrocytic anaemia and dimorphic anaemia. This applies to all ages, even to infancy. Gross splenomegaly, slight jaundice, low fever and anaemia are features of nutritional macrocytic anaemia and of the associated dimorphic anaemia.

If only iron is administered to cases of dimorphic anaemia they may respond well, or moderately, or not at all. Cases which respond well often subsequently recede slightly. If the blood picture is then analyzed again it will be found that often the anaemia has become macrocytic and almost orthochromic and dimorphic anaemia has changed into nutritional macrocytic anaemia. The response of this anaemia to liver alone has been inadequately studied.

The easiest way therefore to ascertain if an anaemia is dimorphic is—malaria being inactive—to give any ferrous salt in adequate dosage and to estimate the first reticulocyte response which almost always rises to its peak about the 9th or 10th day. When this first reticulocyte response has subsided on about the 16th day or even later, liver is given in an adequate dosage and the second reticulocyte response is found to occur about 5 or 6 days after an injection, or about the 10th day after commencing to take liver by the mouth. Only liver injections of the crude extracts can be recommended. Liver extract for injection (B.D.H.) 10–15 c.c. weekly or campolon 15–20 c.c. weekly have always succeeded in the hands of all investigators: in refractory cases the dosage should be doubled. Other more refined extracts, potent in pernicious anaemia, have often proved unreliable unless given in truly enormous dosage.

The reason for this apparent discrepancy is not apparent, but it should be clearly understood by all workers in the subject who, administering a refined liver extract in the dosage adequate for pernicious anaemia, may obtain no response in this anaemia and they may consider that dimorphic anaemia is absent.

Dimorphic anaemia can in the majority of cases be diagnosed on the examination of a well stained and well spread blood smear. That is the only point which the usual clinical worker should grasp. It cannot be diagnosed on the routine blood count, for the colour index is often about unity: it can, however, be diagnosed in the blood smear. The morphology of the blood smear and of the bone marrow confirm the dual deficiency. It is the purpose of this paper to amplify these points in detail.

Technical Matters.

Only unscratched clean slides should be employed, using any of the recognized methods of cleaning: probably the best cleaning fluid is the usual mixture of 10 per cent. potassium bichromate in concentrated sulphuric acid. As this is unobtainable in many parts overseas at the present time, and whereas most of these countries manufacture methylated spirit and rectified spirit the following method can be recommended. *Immediately* after using the slide all immersion oil is removed by cotton wool soaked in any spirit, the slide is placed immediately in ordinary water for a few days, after which the cells are easily removed by rubbing and drying the slide vigorously in a duster. Slides are then stored in rectified spirit, from which they are taken, dried and polished by a clean linen duster immediately before use. Polishing is important and should be vigorous. It ensures a smoother surface and a more even spreading of the blood cells.

The drying of blood films, especially those made from sternal marrow, is often a slow process in the tropics, especially if the humidity is high. At these times many seconds may elapse before the film is dry, especially if the slide has recently been taken from spirit and has been quickly dried. It is then cold and any blood smear spread on it will dry slowly. Further, in very anaemic cases the proportion of serum to cells is so high that the tendency is to spread thick smears lest too few cells are deposited; this, however, prevents rapid drying. Cells which dry slowly are often crenated, or have a pitted surface, whereas those which dry quickly at the extreme edge of the film are well preserved in their shape. Rapid drying is facilitated by polishing the slide and by warming slides by an electric bulb, say that of the microscope lamp. Immediately after sternal puncture I always carry the needle to slides waiting and warmed by the microscope lamp, smears are then spread and the slides are then waved rapidly in the air. It is important to spread smears of the correct thickness, remembering that thin films are spread by retaining a small angle between the two slides during the act of spreading and thus increasing the surface tension. A large angle favours a thick film. No rule can be made concerning the angle required, it will vary from patient to patient and also according to the size of the drop. As a rule very anaemic blood requires a large angle, but normal blood a very small angle.

Any of the Romanowsky stains can be employed; it is much more important to master one stain than to try new stains and new combinations. Almost any stain can be made to give satisfactory results. It is much more important to know how much variation can be produced with the same stain by varying its strength and staining time and differentiation, and to realize how much the normal blood cells may vary in their appearance and to realize how much this may depend on the spreading of the cells on the slide. Two sternal marrow smears may be spread from the same puncture; they may be well spread and may appear almost of similar thickness to the naked eye, they may be stained side by side on the same staining rack for the same period of time and yet they may appear

very dissimilar. This is especially true of the confusion of cells in dimorphic anaemia. Considerable caution is required in the interpretation of any changes.

The Leishman stain probably still is more widely employed than any other stain in the tropics, although it is not in favour with any haematologist of repute, possibly because they are not familiar with it. LEISHMAN designed his stain to demonstrate the malarial parasite, especially the fine subtertian ring. His stain is employed as a very concentrated stain to stain intensely the malarial parasite, but in this concentration it is quite unsuitable to show the finer points of nuclear morphology for it overstains the chromatin network. In fact, after trying many British and German Leishman and Giemsa stains, I must add that I have found none which stained intensely the finer subtertian rings and which did not overstain the nuclear chromatin. All of them in varying degrees, when used in a concentrated manner, stained small rings well and showed the various changes in red cell morphology characteristic of the different malarial infections (Schüffner's, Ziemann's, Maurer's), but then the nuclei were overstained. If used as more dilute stains they demonstrated well nuclear structure, but the finer malarial rings and the other changes in the red cells were faint or even invisible. Usually an intermediate position in which neither parasite nor cell nuclei are overstained is possible, but it is not ideal for either.

Leishman stain can therefore be used to demonstrate fine points in morphology if diluted fairly well. One part of the stain to 1–2 parts of the buffered distilled water is employed and staining is continued for 5 to 10 minutes. A scum and a thick deposit always form and must be washed off vigorously with the buffered water, and the slide differentiated in the buffer for 3 to 5 minutes. By employing dilute stains and by differentiating for a long time little deposit is left on the slide. The great disadvantage of Leishman stain is the thick and adherent deposit which is precipitated when concentrated mixtures are employed for a long time. In addition, after fixing the smear by dropping on the pure stain, it is quite unnecessary to wait before adding the buffer solution for the smear is fixed instantaneously by the methyl alcohol and all delay encourages evaporation and the formation of deposit. Leishman stain is unrivalled as a stain of the polymorphonuclear granules and of the fine subtertian rings. Used as a dilute stain, it defines the chromatin structure of nuclei with a fair degree of precision but tends to overstain the chromatin network. Leishman stain has no fixed chemical composition and all batches of the stain vary; this is a serious disadvantage.

For the definition of nuclear structure a good Giemsa stain is unrivalled. The best results are obtained by employing dilute stains for half an hour or more. I consider R66 or R75 stains* very satisfactory and equal to any Continental stain; the rapid Giemsa made by the same manufacturer has in my hands yielded very unsatisfactory results, giving much deposit and poor nuclear definition. The usual Giemsa stain is not one of fixed nor indeed often of known chemical composition and thus may vary much. By itself it faintly stains haemoglobin, which is usually, together with other cellular structures, stained with Jenner. The Jenner-Giemsa method as set forth in various books on haematology is the best method of staining all the cellular elements in a smear of blood or marrow, especially the latter. It is a long method, taking usually nearly an hour from start to finish. It is shortened only at a price of less efficiency.

In tracing the stages of the developing red cell one is all the time bearing two points in mind, first the structure of the chromatin network, condensing from fine strands to coarse masses, and secondly the development of eosinophilia from basophilia in the cytoplasm. The chromatin network will appear finer in structure if a weak stain is employed or the period of staining is short or the differentiation is continued for a long time. If any of these occur the chromatin network may appear so fine in structure as to resemble that of the megaloblast. Similarly, in my opinion, some, although not all, megaloblasts can be overstained and might then be considered to resemble normoblasts. It might be considered that standardized dilution of stains and times of staining would eliminate this error, but the blood smear itself cannot be standardized and very different effects may be noticed in two smears from the same case stained at the same time by the same stain

* Manufactured by G. T. GURR, London.

in the same way. It is only on a balance of evidence that a cell can, in my opinion, be pronounced megaloblastic. Reference should in any smear always be made to the adult polymorph whose nucleus, cytoplasm and granules afford the best standard by which to judge whether a smear has been overstained or understained and whether the buffer was neutral. It is very important that the buffer should be exactly neutral (pH = 7·0). If a more acid reaction is employed, as advocated by many haematologists, eosinophilia of the cytoplasm is more marked, and it might be considered that premature haemoglobinization of the red cell had occurred or even that the cell was megaloblastic. I employ a solution of the following salts, with which the distilled water at my disposal here has always a reaction of exactly pH = 7·0.

Dihydrogen sodium phosphate ...	0·50 gramme.
Disodium hydrogen phosphate ...	1·50 „
Chloroform	1·0 c.c.
Distilled water to	1 litre.

I.—Normal Erythropoiesis.

There is now almost complete agreement among haematologists concerning the morphology of the cells seen in the later stages of the production of normal red cells; there is, however, no agreement concerning nomenclature nor about the earliest stages of erythropoiesis. The former is not as serious a difficulty as might be supposed although it leads to much confusion. The disagreement concerning the earlier stages of erythropoiesis and leucopoiesis is largely of pure scientific interest and probably will not be settled until improved methods of staining or some totally new method of investigation is found. The disagreement is largely due to the fact that the earliest stages of all primitive red and white cells (polymorphs, eosinophils, basophils, lymphocytes and monocytes) resemble one another more and more as the cell becomes more primitive so that it is extremely difficult, if not impossible, to distinguish them by any Romanowsky stain. Neither can it be certain in any one smear whether any particular primitive cell seen in a certain field of the smear will develop into a leucocyte or an erythrocyte, it is usually argued on a basis of probabilities, namely, that a primitive cell seen in a marrow smear of myeloid leukaemia is probably a myeloblast. It does not follow that it is a normal myeloblast, or that it can be used as a standard by which in a normal marrow smear an unknown primitive cell can be regarded as a myeloblast as opposed to, say, a pro-erythroblast, that is a forerunner of the erythrocytes, especially as these two cells are, according to some observers, almost identical in appearance. In any case one is arguing about cells whose differences are minute, and in any case myeloblasts probably differ considerably among themselves and it is unsatisfactory that they should be identified largely by reference to cells which prevail in the abnormal condition of leukaemia.

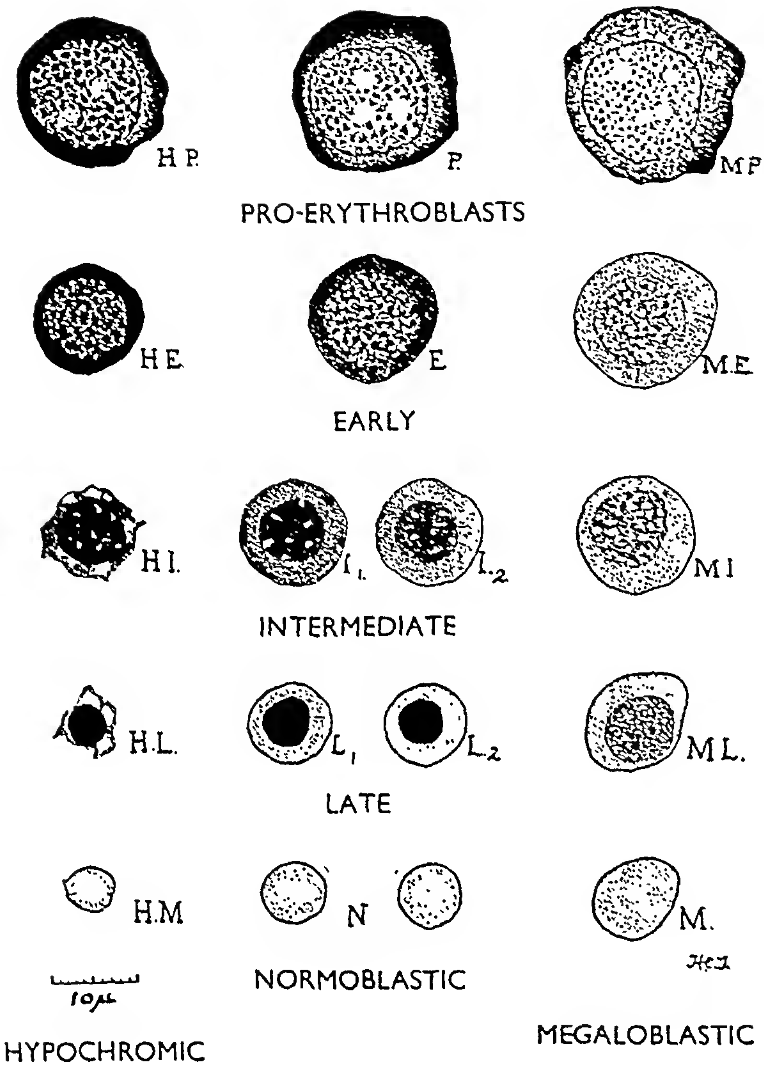
The supra-vital staining elaborated by Doan, Cunningham and Sabin (1925), although making certain contributions to our knowledge of white cell production, has not advanced our knowledge of red cell production. These American investigators have gravely perverted the term megaloblast from its original definition by Ehrlich and Lazarus (1898), who restricted it to the cells seen in the pernicious anaemia group of diseases and to the developing red cells of embryos

under 1 cm. long for these are morphologically indistinguishable (TURNBULL, 1936). The fact that the megaloblast is always a large cell having a large nucleus of fine chromatin network has allowed the majority of American haematologists to use the term megaloblast to describe the most primitive red cell seen in all normal and abnormal marrows. Even large haematological atlases in America (OSGOOD and ASHWORTH, 1937) make no distinction between these two uses of the word megaloblast, and apply this term to cells known by the majority of British and Continental morphologists as the normal primitive red cell (the haemocytoblast or the pro-erythroblast). This misuse of the term megaloblast has been circulated in England by WITTS (1932), WHITBY and BRITTON (1937) and SAMSON WRIGHT (1940) but is not supported in any recent detailed original study on morphology, from British or Continental sources, NAEGELI (1931), SCHULTEN (1937), MALLARMÉ (1937), FERRATA (1933), SEGERADAHL (1935), NORDENSEN (1935), ROHR (1935), TURNBULL (1936), ISRAËLS (1939), SCOTT (1939), and by occasional American authors, such as JONES (1934). The position adopted here is that of the latter group, namely, that a pathological development of red cells occurs in the pernicious anaemia group of blood disorders, and that this is megaloblastic. The normal production of cells as occurring in healthy marrow is called here normoblastic (see Diagram, p. 163) and produces normocytes. The abnormal development of hypochromic cells, often microcytes, in iron-deficiency anaemias is referred to as hypochromic development and has been but little studied in haemotology: STODTMEISTER (1937) and SCOTT (1939) described it in detail. In dimorphic anaemia there is a mixture of hypochromic and megaloblastic development together with much normoblastic development.

It is now necessary to define the terms employed. Unfortunately, it is not possible at the present time to arrange for the production of truthful coloured plates, and misleading coloured plates would only confuse the issue. The terms employed are defined in terms of written descriptions and of black and white drawings, which portray the cells more in the spirit of a diagram than in the spirit of a picture. The terms employed are modelled on the careful studies by SCHULTEN (1937), DAMESHEK and VALENTINE (1937) and ISRAËLS (1939 and 1941), all of whom have published illustrations. For reasons already discussed the earliest stages are omitted, because I am unable either to subscribe or to deny the views set forth by other haematologists. I prefer to start with the pro-erythroblast and not with the debatable haemocytoblast, a term used in different ways by different authors (ISRAËLS 1939, TURNBULL 1936, and FERRATA 1938). The earliest primitive red cell concerning which I feel reasonably certain is the pro-erythroblast.

There are certain broad changes which occur in the development of red cells which it is well to bear in mind.

1. The chromatin network at first fine in structure condenses into larger and larger masses until a structureless mass is formed. This is then usually extruded. The nucleus of the early cells is also stained a pinkish transparent colour and its tone is pale but as it matures it is stained a deeper and deeper



H.P. Hypochromic pro-erythroblast.
H.E. Hypochromic early normoblast.
H.I. Hypochromic intermediate normoblast.
H.L. Hypochromic late normoblast.
H.M. Hypochromic microcyte.
P. Pro-erythroblast (normal).
E. Early normoblast (normal).
I_1 Intermediate normoblast (normal), much basophilia.
I_2 Intermediate normoblast (normal), much polychromasia.
L_1 Late normoblast (normal), much polychromasia.
L_2 Late normoblast (normal), eosinophilia.
N. Normocyte.
M.P. Megaloblastic pro-erythroblast (N.M.A.).
M.E. Early megaloblast. (N.M.A.)
M.I. Intermediate megaloblast. (N.M.A.)
M.L. Late megaloblast. (N.M.A.)
M. Macrocyte (N.M.A.)

DIAGRAM

Hypochromic development in iron deficiency anaemia.
Normoblastic development in normal marrow.
Megaloblastic development in nutritional macrocytic anaemia.
Jenner-Giemsa Stain. × 1000

The differences are most marked at the late and intermediate stages and are of doubtful validity at the most primitive stages. All varieties are seen in dimorphic anaemia, hypochromic cells in the central portions of the marrow smear, megaloblastic cells at the extreme tail.

under 1 cm. long for these are morphologically indistinguishable (TURNBULL, 1936). The fact that the megaloblast is always a large cell having a large nucleus of fine chromatin network has allowed the majority of American haematologists to use the term megaloblast to describe the most primitive red cell seen in all normal and abnormal marrows. Even large haematological atlases in America (OSGOOD and ASHWORTH, 1937) make no distinction between these two uses of the word megaloblast, and apply this term to cells known by the majority of British and Continental morphologists as the normal primitive red cell (the haemocytoblast or the pro-erythroblast). This misuse of the term megaloblast has been circulated in England by WITTS (1932), WHITBY and BRITTON (1937) and SAMSON WRIGHT (1940) but is not supported in any recent detailed original study on morphology, from British or Continental sources, NAEGELI (1931), SCHULTEN (1937), MALLARMÉ (1937), FERRATA (1933), SEGERADAHL (1935), NORDENSEN (1935), ROHR (1935), TURNBULL (1936), ISRAËLS (1939); SCOTT (1939), and by occasional American authors, such as JONES (1934). The position adopted here is that of the latter group, namely, that a pathological development of red cells occurs in the pernicious anaemia group of blood disorders, and that this is megaloblastic. The normal production of cells as occurring in healthy marrow is called here normoblastic (see Diagram, p. 163) and produces normocytes. The abnormal development of hypochromic cells, often microcytes, in iron-deficiency anaemias is referred to as hypochromic development and has been but little studied in haemotology: STODTMEISTER (1937) and SCOTT (1939) described it in detail. In dimorphic anaemia there is a mixture of hypochromic and megaloblastic development together with much normoblastic development.

It is now necessary to define the terms employed. Unfortunately, it is not possible at the present time to arrange for the production of truthful coloured plates, and misleading coloured plates would only confuse the issue. The terms employed are defined in terms of written descriptions and of black and white drawings, which portray the cells more in the spirit of a diagram than in the spirit of a picture. The terms employed are modelled on the careful studies by SCHULTEN (1937), DAMESHEK and VALENTINE (1937) and ISRAËLS (1939 and 1941), all of whom have published illustrations. For reasons already discussed the earliest stages are omitted, because I am unable either to subscribe or to deny the views set forth by other haematologists. I prefer to start with the pro-erythroblast and not with the debatable haemocytoblast, a term used in different ways by different authors (ISRAËLS 1939, TURNBULL 1936, and FERRATA 1938). The earliest primitive red cell concerning which I feel reasonably certain is the pro-erythroblast.

There are certain broad changes which occur in the development of red cells which it is well to bear in mind.

1. The chromatin network at first fine in structure condenses into larger and larger masses until a structureless mass is formed. This is then usually extruded. The nucleus of the early cells is also stained a pinkish transparent colour and its tone is pale but as it matures it is stained a deeper and deeper

purple, is less transparent and its tone becomes much darker. A considered opinion on all these points affords the best indication of the age of the cell.

2. The cytoplasm at first a deep blue colour and dark in tone, matures by the development of eosinophilia, the disappearance of basophilia and a progressive loss of tone and increase of transparency.

3. These two changes of nuclear condensation and of cytoplasmic ripening normally march together with only slight variation.

4. If the cytoplasm has developed more eosinophilia than the nuclear network condensation would justify, this dissimilarity of development is megaloblastic and suggestive of a liver principle deficiency.

5. If the cytoplasm develops eosinophilia poorly, remains scanty in amount but the nuclear network has condensed much, this dissimilarity of development is hypochromic and suggestive of iron deficiency.

The following notes on the morphology of the blood in dimorphic anaemia are based principally on an analysis of sixty-two cases of macrocytic hypochromic anaemia in all of whom the sternal puncture smear and the peripheral blood smear were stained and examined by myself. The views held were amplified by reference to some thirty cases of normocytic hypochromic anaemia in which it was considered dimorphic anaemia was certainly present.

These ninety-two cases are selected from a total number of 508 in-patient cases of anaemia, in some 300 of whom the sternal dry-smear was examined, because although in many of these 508 cases was dimorphic anaemia present, yet only 90 cases fulfilled all the necessary conditions of adequate investigation of cell volume, cell diameter, test meal, van den Bergh, response to treatment and of length of stay in hospital.

The appearance of the normal marrow dry-smear was studied in some eight cases having a peripheral blood count falling within the normal range of PRICE-JONES, VAUGHAN and GODDARD (1935). The descriptions refer to cells stained by the lengthy but accurate Jenner-Giemsa method.

1. *Pro-erythroblast.* Average size 18 (14) μ,* range 15–22 (12–15) μ*

Nucleoli.—Two or more are always present, but are seen and counted with difficulty. They appear as pale areas in the chromatin network.

Nuclear chromatin network.—Very fine in texture, finely stippled, no condensation into masses or nodes, very pale in tone, much paler than that of the surrounding cytoplasm. Colour is pinkish-red. No coalescence of chromatin network at edges of nucleus so that no clear nuclear edge or membrane can be seen.

Cytoplasm.—Very dark in tone, much darker than that of the nucleus. Colour is deep blue like Fehling's solution, and opaque in texture. Often a small paler perinuclear zone. Often blunt pseudopodial protrusions at the margin.

2. *Early normoblast.* Average size 14·5 (11·5) μ, range 12–18 (10–14) μ.

Nuclear chromatin network.—Definite but slight condensation into small masses or nodes, but the general pattern is still fine. Nucleus is smaller in size, darker in tone and a deeper crimson red colour than that of the preceding cell. No nucleoli.

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* Here, as elsewhere; the convention is followed of giving first and unbracketed the dimensions of the cell, followed by those of the nucleus, the latter alone being given within brackets.

those cases which reacted well to liver. The megaloblastic change varied considerably from case to case and was usually most severe in severely anaemic cases. Descriptions refer to cases in which the changes were marked and rather exaggerate the changes found in the usual case.

Primitive unclassified cell.

Towards the tail and edges of many sternal smears of N.M.A. are seen cells which I am unable to classify. They appear to be primitive cells, possibly derived from, or a part of, the reticulo-endothelial system. They are far too numerous and too constant in structure to be artifacts.

Nucleus.—Average size 16 μ, range 13–22 μ. A coarsely speckled chromatin network, very reticular and loose in its pattern, often but not always with nucleoli, almost always oval in outline. Stains crimson red. The structure is more coarse than that of the pro-erythroblast.

Cytoplasm.—Usually none, or ill-defined and scanty, pale, transparent blue cytoplasm is found. Some have a granular cytoplasm and appear phagocytic engulfing large masses of malarial pigment. Often occurring in groups, the cytoplasmic mass appears undivided and common to several nuclei. At other times the cytoplasm appears drawn out into long streaks and threads. It is not clear whether this primitive cell ever develops into the pro-erythroblast.

1. *N.M.A. Pro-erythroblast.* Average size 20 (14) μ, range 17–24 (13–18) μ.

Nucleoli.—Usually several, 3–5, but seen and counted with difficulty.

Nuclear chromatin network.—Very fine in texture and stippling, very pale, and very transparent. All these changes are slightly more marked than in the normal pro-erythroblast but the differences are but slight and are debatable.

Cytoplasm.—Almost never a deep blue, but paler in colour; all shades are seen until some are sky-blue, like the " classical " myeloblast. Often has a foamy, almost vacuolated, appearance. A narrow rim at the extreme edge is often much darker in colour.

All stages are seen between this cell, showing much megaloblastic change, and a normal pro-erythroblast.

2. *Early N.M.A. megaloblast.* Average size 15 (11·5) μ, range 12–19 (8–15) μ.

No nucleoli.

Nuclear chromatin network.—The network shows less signs of condensation of the chromatin network than in that of the normal early normoblast, so that it remains finer in texture, paler in colour and more transparent. The differences are, however, but slight.

Cytoplasm.—Varies between a moderately deep blue to a pale sky-blue, even a faintly polychromasic colour may be seen. Some foaminess of texture may be visible. It is almost always decidedly paler and more polychromasic than the cytoplasm of the normal early normoblast.

All stages are seen between this cell showing much megaloblastic change and a normal early normoblast.

3. *Intermediate N.M.A. megaloblast.* Average size 12 (9) μ, range 9–15 (7–11) μ.

Nuclear chromatin network.—The nucleus shows definite signs of developing as compared to that of the preceding cell, but it is definitely more reticular in its structure than that of the normal cell. In the latter there has been so much confluence of the chromatin strands that the meshes are few and relatively small in area so that it resembles a target shot through by small bullet holes. The nucleus of the intermediate N.M.A. megaloblast resembles a network of coarse ropes. It is not as fine in structure or as reticulated or as large or as pale in its staining as the P.A. intermediate megaloblast.

Cytoplasm.—Much variation. Some are a pale transparent sky-blue with no eosinophilia; the majority are a pale, transparent, light purple (polychromasia), a minority are a deeper purple colour. The cytoplasm is more scanty in appearance and not so eosinophilic or as pale as in the P.A. intermediate megaloblast.

Cytoplasm.—Almost identical in tone, colour and opacity to the deep blue of the pro-erythroblast. No perinuclear zone of pallor. No pseudopodial protrusions. The tone of the nucleus and that of the cytoplasm are now equal. Both are showing tinges of purple either in the red of the nucleus or in the blue of the cytoplasm.

3. *Intermediate normoblast.* Average size 12 (7) μ, range 9–14 (5–10) μ.
Nuclear chromatin network.—Moderate condensation into large masses, which are tending to become confluent and whose pattern is very coarse. In the meshes of the network are pale hole-like areas like holes shot in a target (cart-wheel nucleus). The nucleus is smaller in size and also darker in tone, and a deeper purple colour than that of the preceding cell. Its tone usually exceeds that of the cytoplasm.
Cytoplasm.—Much variation. A minority show no change from that of the preceding cells, the majority show loss of tone, loss of opacity, decrease of depth of blueness (basophilia), and the commencement of a purple colour with the advent of some eosinophilia, a minority may show this change fairly well advanced (polychromasia). Almost always the colour is more transparent than that of the preceding cell.

4. *Late normoblast.* Average size 9 (5) μ, range 6–12 (4–8) μ.
Nuclear chromatin network.—Complete condensation has either occurred, and no structure is visible, or this has almost completely occurred and a very faint structure can still be discerned. Its size is now small, its tone is very dark, its colour a purplish-black.
Cytoplasm.—The majority show much development of eosinophilia, and are purple in colour, transparent in consistency, and very light in tone (polychromasic), a minority show complete eosinophilia with no trace of polychromasia, a very small minority may still show much basophilia.

All intermediate stages are seen, and it is obviously impossible to define exactly when a cell passes from one stage to another. As the majority of cells actually are at some such intermediate stage, it follows that one haematologist might consider that one particular cell was more nearly that of, say, the intermediate stage but another would consider that it had developed enough to justify inclusion as a late normoblast. The rigid classification and enumeration of cells into various categories conveys a specious impression of accuracy to a subject which can only remain a matter of opinion. Further, not only are there variations in a vertical direction with all degrees of development of the normoblast but also there is a fairly wide range of normal variation among cells of the same degree of maturation of the nucleus. Thus a late normoblast whose nucleus has still faint evidence of structure may show complete eosinophilia, or polychromasia, and a few may show a fair degree of purplish basophilia. I have only been able to study normal erythropoiesis in some eight Africans and would hesitate to say exactly the extent of the normal range. This appears to me of considerable importance for the variation which is found within the normal marrow smears seems to extend (diagrammatically) to the right until it meets the megaloblastic erythropoiesis of nutritional macrocytic anaemia. I am unable to draw any clear dividing line.

II.—Megaloblastic Erythropoiesis in Nutritional Macrocytic Anaemia.

These observations are based on a study of the marrow smears from thirty-one cases of macrocytic orthochromic anaemia. Reliance was placed on

those cases which reacted well to liver. The megaloblastic change varied considerably from case to case and was usually most severe in severely anaemic cases. Descriptions refer to cases in which the changes were marked and rather exaggerate the changes found in the usual case.

Primitive unclassified cell.

Towards the tail and edges of many sternal smears of N.M.A. are seen cells which I am unable to classify. They appear to be primitive cells, possibly derived from, or a part of, the reticulo-endothelial system. They are far too numerous and too constant in structure to be artifacts.

Nucleus.—Average size 16 μ, range 13–22 μ. A coarsely speckled chromatin network, very reticular and loose in its pattern, often but not always with nucleoli, almost always oval in outline. Stains crimson red. The structure is more coarse than that of the pro-erythroblast.

Cytoplasm.—Usually none, or ill-defined and scanty, pale, transparent blue cytoplasm is found. Some have a granular cytoplasm and appear phagocytic engulfing large masses of malarial pigment. Often occurring in groups, the cytoplasmic mass appears undivided and common to several nuclei. At other times the cytoplasm appears drawn out into long streaks and threads. It is not clear whether this primitive cell ever develops into the pro-erythroblast.

1. *N.M.A. Pro-erythroblast.* Average size 20 (14) μ, range 17–24 (13–18) μ.

Nucleoli.—Usually several, 3–5, but seen and counted with difficulty.

Nuclear chromatin network.—Very fine in texture and stippling, very pale, and very transparent. All these changes are slightly more marked than in the normal pro-erythroblast but the differences are but slight and are debatable.

Cytoplasm.—Almost never a deep blue, but paler in colour ; all shades are seen until some are sky-blue, like the " classical " myeloblast. Often has a foamy, almost vacuolated, appearance. A narrow rim at the extreme edge is often much darker in colour.

All stages are seen between this cell, showing much megaloblastic change, and a normal pro-erythroblast.

2. *Early N.M.A. megaloblast.* Average size 15 (11·5) μ, range 12–19 (8–15) μ.

No nucleoli.

Nuclear chromatin network.—The network shows less signs of condensation of the chromatin network than in that of the normal early normoblast, so that it remains finer in texture, paler in colour and more transparent. The differences are, however, but slight.

Cytoplasm.—Varies between a moderately deep blue to a pale sky-blue, even a faintly polychromasic colour may be seen. Some foaminess of texture may be visible. It is almost always decidedly paler and more polychromasic than the cytoplasm of the normal early normoblast.

All stages are seen between this cell showing much megaloblastic change and a normal early normoblast.

3. *Intermediate N.M.A. megaloblast.* Average size 12 (9) μ, range 9–15 (7–11) μ.

Nuclear chromatin network.—The nucleus shows definite signs of developing as compared to that of the preceding cell, but it is definitely more reticular in its structure than that of the normal cell. In the latter there has been so much confluence of the chromatin strands that the meshes are few and relatively small in area so that it resembles a target shot through by small bullet holes. The nucleus of the intermediate N.M.A. megaloblast resembles a network of coarse ropes. It is not as fine in structure or as reticulated or as large or as pale in its staining as the P.A. intermediate megaloblast.

Cytoplasm.—Much variation. Some are a pale transparent sky-blue with no eosinophilia ; the majority are a pale, transparent, light purple (polychromasia), a minority are a deeper purple colour. The cytoplasm is more scanty in appearance and not so eosinophilic or as pale as in the P.A. intermediate megaloblast.

All stages are seen between this cell showing much megaloblastic change and a normal intermediate normoblast.

4. *Late N.M.A. megaloblast.* Average size 10 (7·0) μ, range 9–14 (5–8) μ.

Nucleus.—Definite structure is always easily visible, the nucleus appears like a semi-transparent woollen garment, the filaments of the network being still relatively fine in texture. They are now largely confluent, yet they are quite distinct from the large structureless masses which condense to form the fully pyknotic nucleus of the normal late normoblast. The nucleus is usually eccentric and is extruded before it is fully pyknotic. *Complete pyknosis is rarely seen.*

Cytoplasm.—Complete eosinophilia is rare but does occur; the majority of cells are a pale transparent polychromasia. The nucleus is often extruded while the cytoplasm is still polychromasic. The cell is often markedly oval in shape.

All stages are seen between this cell, showing much megaloblastic change and the normal late normoblast.

III.—Hypochromic Erythropoiesis of Iron Deficiency.

This has received far less study than the changes in megaloblastic development. Detailed accounts are given by Stodtmeister (1937) and Bodley Scott (1939), but certain points of difference are maintained here.

A study has been made of the dry marrow smear of eight cases of microcytic hypochromic anaemia.

1. *Hypochromic pro-erythroblast.* Average size 17 (13) μ, range 14–20 (11–14) μ.

Nucleoli.—Two or more, counted with great difficulty.

Nuclear chromatin network.—Stains possibly more darkly, and is of coarser structure, than that of the normal pro-erythroblast, but this change is debatable.

Cytoplasm.—Very dark blue, very opaque, little, if any, perinuclear pallor. The differences between this and the normal cell are but slight, and are largely one of size. Pro-erythroblasts are usually consistently smaller in hypochromic anaemia.

2. *Hypochromic early normoblast.* Average size 12 (10) μ, range 10–15 (8–12) μ.

Nuclear chromatin network.—More condensed, stains more darkly and coarser in structure than that of the normal cell.

Cytoplasm.—Dark blue, as in the normal early normoblast.

The differences between this and the normal cell are largely one of size and are indeed but doubtful.

3. *Hypochromic intermediate normoblast.* Average size 10·5 (7) μ, range 8–12 (6–9) μ.

Nuclear chromatin network.—More condensed, and coarser in structure than that of the normal intermediate normoblast and is fast becoming pyknotic.

Cytoplasm.—A fair number still show deep basophilia, the majority show commencing eosinophilia and are a deep, opaque purple colour, a minority are a more pale and transparent purple, none are pale and transparent enough to be classified as polychromasic. The cytoplasm is often scanty, it may be vacuolated or have a foamy appearance, the cell margin is irregular and has a ragged outline.

4. *Hypochromic late normoblast.* Average size 7 (5) μ, range 5–10 (3–8) μ.

Nucleus.—Completely pyknotic, often clover leaf in pattern due presumably to fragmentation, a rare phenomenon in N.M.A. although it may occur. Extrusion of the nucleus is more common than fragmentation even in hypochromic anaemia.

Cytoplasm.—Scanty, often having large unstained areas around the nucleus, a fair number show eosinophilia, others show polychromasia, some even show much dark basophilia. The cell margin is very ragged.

The significance of the megaloblastic development in nutritional macrocytic anaemia and of the hypochromic development in iron-deficiency anaemia.

Few reports in any detail have been given of the changes in the bone marrow dry smear in nutritional macrocytic anaemia. Thus FOY and KONDI (1939) allude briefly to megaloblastic changes in nutritional macrocytic anaemia being always present in their cases and HAMILTON FAIRLEY *et al.* (1938) found both normoblastic and megaloblastic changes. The latter also stressed the fact that the completely developed eosinophilic megaloblast " with a finely stippled nucleus was a rarity and that cells with this degree of ripening almost invariably showed some clumping of the reticulum." They " suggest that failure of maturation of the megaloblast only accounted for part of the pathological changes."

In the allied pernicious anaemia of pregnancy seen in temperate regions UNGLEY (1938) has noted that maturation is usually of the normal type and seldom of the megaloblastic variety. In my opinion this anaemia of pregnancy is identical with nutritional macrocytic anaemia. Professor TURNBULL (see discussion after HAMILTON FAIRLEY *et al.* (1938)) has examined the bone marrow in Dr. LUCY WILLS'S rhesus monkeys who had contracted nutritional macrocytic anaemia as a result of dietetic restrictions and he thought the most characteristic changes were in the pathological promyelocytes and not in the red cell series. Presumably megaloblastic change was not marked. No illustrations have been, as far as I am aware, published on the megaloblastic changes in nutritional macrocytic anaemia.

The present studies offer certain definite distinctions between the megaloblasts seen in nutritional macrocytic anaemia and those seen in pernicious anaemia. The definition and dimensions of the P.A. megaloblasts is in accordance with the views of BODLEY SCOTT (1939) and ISRAËLS (1939).

To summarize: The megaloblast of pernicious anaemia shows always a much more reticular or primitive type of chromatin network and yet a more eosinophilic or mature cytoplasm than that of the megaloblast of nutritional macrocytic anaemia. In addition the P.A. megaloblast is larger in diameter. This is due, morphologically speaking, to the fact that the megaloblast of pernicious anaemia is developing into a completely eosinophilic macrocyte, having an increased diameter but no increased cell thickness. The reverse occurs in nutritional macrocytic anaemia. This change in thickness is not obvious in the dry smear, which can only assess an increase in cell diameter. N.M.A. megaloblasts appear more compact, smaller, more like a normoblast than those of pernicious anaemia.

The N.M.A. megaloblasts approximate more to the normoblasts. Many will consider that they are normoblasts but on the evidence before me I would maintain that they can be usually distinguished in at least their later stages on the two following points. First, there is always uneven development of the cytoplasm and of the nucleus, when the former is nearly mature and shows much polychromasia and pallor the latter is still as reticular as that of the

intermediate or early normoblast. Secondly, the nucleus never matures fully, complete pyknosis is never seen, some reticulation is always visible. Even when the nucleus is on the point of extrusion and has become extremely eccentric it still retains much structure and is larger in size than that of the late normoblast.

Much erythropoiesis in pernicious anaemia and in nutritional macrocytic anaemia is normoblastic. Although in pernicious anaemia megaloblastic erythropoiesis usually predominates, yet normoblastic erythropoiesis may predominate (BODLEY SCOTT, 1939). In nutritional macrocytic anaemia normoblastic erythropoiesis vastly predominates and N.M.A. megaloblasts

Stage.	Nutritional Macrocytic Anaemia.	Pernicious Anaemia.
Late megaloblast.		
Diameter of cell ...	10·0 μ	10·0 μ
" of nucleus ...	7·0 μ	5·0 μ
Cytoplasm	Polychromasic	Eosinophilic.
Chromatin	Slight reticulation	Much reticulation
Intermediate megaloblast.		
Diameter of cell ...	12·0 μ	15·5 μ
" of nucleus ...	9·0 μ	12·0 μ
Cytoplasm	Dark polychromasia	Pale polychromasia
Chromatin	Slight reticulation	Much reticulation
Early megaloblast.		
Diameter of cell ...	15·0 μ	20·0 μ
" of nucleus ...	11·5 μ	14·0 μ
Cytoplasm	Pale purple	Very pale basophilia
Chromatin	Slight reticulation	Much reticulation

may only be visible at the extreme tail of the smear. Once again I wish to emphasize that all stages between normoblastic erythropoiesis and megaloblastic erythropoiesis can be found in nutritional macrocytic anaemia.

When one ponders what constitutes the essential difference between the morphology of megaloblastic development and of normoblastic development one is forced to conclude that it consists in the relative paucity of the maturation of the nucleus. In the relative or complete absence of the liver principle in nutritional macrocytic anaemia or pernicious anaemia a varying number of pro-erythroblasts cannot mature normally, the chromatin network remains open and reticular, the nucleus and the cell remain large. The cytoplasm, however, can and does develop pallor and eosinophilia, a change for which iron appears mainly responsible and there is no iron deficiency in these anaemias.

Nothing is known concerning the significance of the differences between the P.A. megaloblasts and the N.M.A. megaloblasts ; it appears unwise to speculate on these changes until they are confirmed by other observers. Further, this deficiency of the liver principle probably operates at all stages of the developing red cell and not just at one stage as some have supposed.

Iron appears essential for the development of haemoglobin and eosinophilia in the cytoplasm. In its relative absence the cytoplasm remains basophilic or darkly polychromasic but the nucleus matures, for the liver principle is present, until it is a small pyknotic mass. The cytoplasm develops eosinophilia poorly, so that the cell margins are irregular and its contents small in amount and vacuolated in appearance. Extrusion of the small nucleus leaves a hypochromic microcyte. Iron probably produces changes at all stages of development and not just at the last stages as some would suggest.

These two changes are largely complementary. The liver principle is essential to nuclear maturation ; in its absence there is a " shift to the right " (see Diagram, p. 163) ; cytoplasmic ripening appears excessive as compared with nuclear maturation ; iron is essential for cytoplasmic development, in its absence there is a " shift to the left " and nuclear maturation appears excessive as compared with cytoplasmic maturation. In the absence of both substances there is an equal failure of development of the nucleus and of the cytoplasm, but the cell is not " shifted " either to the right or the left but appears to remain within the normoblastic series. I trust this hypothesis does not appear too ingenious ; in any case in dimorphic anaemia normoblastic erythropoiesis vastly predominates although some hypochromic and megaloblastic development can be found. NAPIER and MAJUMDA (1937) noted no megaloblastic changes in their cases of dimorphic anaemia. I consider the changes are definite but are found in only a minority of cells at the tail of the smear.

ERYTHROPOIESIS IN DIMORPHIC ANAEMIA.

The bone marrow smear in dimorphic anaemia shows megaloblastic, normoblastic and hypochromic erythropoiesis. If nutritional macrocytic anaemia deficiency predominates and iron deficiency is slight the marrow smear approximates to that of nutritional macrocytic anaemia, and the following points are noted :—

1. Increased cellularity of the marrow smear.
2. Large numbers of early cells, " primitive cells " at the tail, pro-erythroblasts, early megaloblasts and early normoblasts ; more than in any other anaemia except those of the pernicious anaemia group, and many more of these cells are seen than in normal marrow, in which all early red cell precursors are very rare.
3. Increased number of red cell precursors as compared to white cell precursors. In normal marrow this proportion is usually about 1 : 5 ; in this

variety of anaemia it may be as low as 1 : 1; occasionally red cell precursors predominate.

4. All stages between megaloblastic and normoblastic development are seen but a minority show definite megaloblastic change and a fair number of megaloblasts always predominate towards the tail and edges of the smear.

5. Normoblastic cells form the large majority of developing cells.

6. Hypochromic erythropoiesis is slight and may not be detected. It is best seen in the central portions of the smear.

7. The red cells of the marrow smear show much polychromasia, increased anisocytosis, and slight or absent hypochromia. Macrocytes are more obvious towards the tail of the smear and hypochromia towards the centre.

If, on the other hand, iron deficiency predominates over that of nutritional macrocytic anaemia, then the following changes are seen :—

1. Increases cellularity.

2. Early red cell precursors are not as numerous as in the preceding group, but some increase does occur.

3. The relative proportion of red cell precursors compared to white cell precursors is increased.

4. Hypochromic erythropoiesis is seen in a fair number of cells in the central portions of the smear.

5. Normoblastic erythropoiesis is variable but is usually the predominating type.

6. Megaloblastic erythropoiesis is very scanty and may be completely absent. It should be searched for at the extreme tail of the smear where usually a few late megaloblasts are seen. In other cases the changes are not so definite and only megaloblastic tendencies can be recognized.

7. The red cells of the marrow smear show some polychromasia. Much hypochromia and some microcytosis are seen in the central portions of the smear, anisocytosis and well stained cells, some macrocytic, are seen towards the tail.

An intermediate group in which both deficiencies are approximately equal shows changes which lie between those already described.

No attempt here is made to describe the changes which occur in the white cell precursors, these vary largely according to what intercurrent diseases are present, such as hookworms, malaria and so on. The senile pernicious anaemia neutrophil cell and pathological large metamyelocytes are sometimes but by no means always visible.

Differential cell counts on the marrow smears.

It follows from what has gone before that it is almost impossible to publish differential cell counts on marrow smears. It is, in my opinion, impossible to do so for the following reasons :—

1. Vertical variation (see Diagram) is wide, and does not admit of sharp definitions into definite categories ; the latter may in their broad outline be necessary for descriptive purposes, but intermediate categories always predominate and are listed by different observers in different groups as an expression of individual opinion.

2. Lateral variation is wide. No clear line separates normoblastic cells and those with slight " shift to the right " and some megaloblastic tendency as in nutritional macrocytic anaemia from those with marked megaloblastic tendencies as in pernicious anaemia. There is thus a fairly large group which, in my opinion, would be regarded by some as normoblasts, by others as megaloblasts.

3. In dimorphic anaemia the spreading of the cells in the marrow smear is very uneven. Small hypochromic microcytes and their precursors tend to collect in the head and central portions of the smear, large orthochromic macrocytes and their precursors tend to collect in the tail and at the edges.

For all these reasons differential counts on some 100–200 cells afford little indication of what is occurring in different parts of the smear, and conveys an element of false accuracy. They have been performed in some fifty cases. I now consider the numbers to be so inaccurate as not to warrant publication. It appears to me more necessary to decide what types of erythropoiesis are present.

Morphology of the Peripheral Blood.

Amidst all the conflict of words and illustrations which surrounds the question of marrow cell morphology the busy clinical worker may ask whether it will ever be possible to decide quickly if dimorphic anaemia is present. The answer is plain : the majority of cases can be diagnosed with certainty on an examination of a thinly spread film of the peripheral blood, stained by any Romanowsky stain, but preferably by a Jenner, or a Jenner-Giemsa method.

In the central portions the picture is one of iron-deficiency. Hypochromic cells abound, a few are definitely microcytic and less than 5 μ in diameter. Anisocytosis is but slight. A few macrocytes, that is cells exceeding 9 μ in diameter, may be seen ; sometimes they are well stained and full of haemoglobin and at other times they are markedly hypochromic. Poikilocytes may be common.

On examining the tail and edges of the film one is struck at once by a great change in appearance. Many can scarcely believe they are looking at the same blood slide, for the cells are all well stained, none are hypochromic ; these well stained cells are often oval, they show no central pallor, a few, but not many, are macrocytic and exceed 9 μ in diameter. Some of the macrocytes may be hypochromic. Polychromasia is present and may be very marked.

It is this dual picture which suggests more clearly and more correctly than anything else the dimorphic nature of the deficiency.

If, however, one deficiency is severe but the other is but slight then the blood smear may only show the appearance of the major deficiency. Thus, if iron deficiency is but slight, the blood smear may resemble entirely that of nutritional macrocytic anaemia, but a few hypochromic cells will usually be seen in the central portions of the smear. Again, if the deficiency of nutritional macrocytic anaemia is but slight the blood smear may resemble entirely that of iron deficiency but a few macrocytic oval cells will usually be seen at the extreme tail of the smear. It is therefore absolutely essential to search the smear from head to tail bearing in mind that even in normal blood the smaller cells tend to collect at the head and the larger ones tend to be more numerous at the tail. This change is much more marked in dimorphic anaemia than in any other anaemia.

If iron alone is given to a case of dimorphic anaemia the majority show definite signs of improvement but a few show no signs of improvement in the blood count. Some cases regenerate, albeit slowly, until the haemoglobin has reached a normal level; red cells regenerate slowly in number and after a time tend in a fair number of cases to become stationary in number at a sub-optimal figure. If the blood smear is then examined the picture is usually that of uncomplicated nutritional macrocytic anaemia, and the hypochromia has disappeared. The mean cell volume at this stage is almost always markedly macrocytic, but mean corpuscular haemoglobin concentration has increased almost to a normal orthochromic figure. If liver is given at this stage a second, possibly feeble, reticulocyte crisis occurs, being slight if the red cell count is high, but more if the red cell count is low, and red cells regenerate more rapidly in number. The mean cell volume then drops to a normal figure under the influence of liver.

In almost all severely anaemic cases late N.M.A. megaloblasts and late normoblasts can be found in the peripheral blood smear if a thorough search is made. They resemble the cells described under these names in the bone marrow and are not described again, except to stress the point that intermediate types between typical normoblasts and typical N.M.A. megaloblasts are frequently seen and again that the fully matured eosinophilic cell with a marked reticular chromatin network in the nucleus (the classical megaloblast of Ehrlich) is rare.

Polychromasia, especially in the macrocytic cells, is common in those cases which show a reticulocytosis. Its detection depends much on which stain is used; in my opinion it is much more marked with Leishman than with Giemsa stains, and requires that the buffer be exactly neutral for its precise detection, an acid buffer minimizing the change, an alkaline buffer accentuating the change. Punctate basophilia is fairly common, and is brought out by overstaining, especially with Leishman. Howell-Jolly bodies and Cabot rings may occasionally be seen. Poikilcytes may be very common.

If hypochromic macrocytes occur, as are indeed common, these cells appear

to suffer distortion easily in the process of fixing and staining, so that their contents may appear vacuolated, and irregular in texture, and their edges pitted and jagged. Cases with severe anaemia in which the proportion of serum to cells is much increased present many difficulties in even spreading and in rapid drying, much distortion and many artifacts occur.

Senile pernicious anaemia neutrophils are occasionally seen. There is a total neutropenia in many cases. Eosinophil cells, sometimes containing vacuoles, are increased in the majority but by no means in all cases, harbouring hookworms and other helminths. Monocytes, often with young round nuclei, and amoeboid pseudopodia and containing malarial pigment, are seen in increased numbers in the recovery stage of malaria. Platelet counts are decreased to 100,000 or below and purpura is common.

Summary.

1. The modern approach to anaemia consists in stressing the deficiency aspect of anaemia even when this deficiency is occasioned by, or increased in, a certain disease. Anaemia is seldom regarded as entirely secondary to disease unless this disease causes severe haemolysis.

2. This outlook has not been applied to the classification of anaemia in the tropics, where anaemia is regarded as due almost exclusively to the presence of tropical diseases.

3. Most cases of anaemia in Uganda natives show evidence of a dual deficiency of iron and of that present in nutritional macrocytic anaemia.

4. It is suggested that this should be called dimorphic anaemia.

5. 174 cases of anaemia in Uganda natives were examined by estimation of M.C.V., M.C.H.C., peripheral blood counts, sternal puncture, test meal, van den Bergh reaction and the reticulocyte response to iron and to liver.

6. 100 of these cases appeared to be cases of dimorphic anaemia.

7. The commonest cause of severe iron deficiency in dimorphic anaemia appeared to have been a heavy hookworm load and a diet deficient in iron.

8. The commonest cause of the nutritional macrocytic anaemia type of deficiency appeared to have been due to a diet poor in meat and possibly in green vegetables.

9. The sternal dry smear has been examined in 162 of these 174 cases and in some 145 other cases of anaemia and the megaloblastic tendencies in erythropoiesis in nutritional macrocytic anaemia and the hypochromic regeneration in iron-deficiency anaemia are described in detail and compared with normal erythropoiesis studied in eight normal Africans.

10. In dimorphic anaemia all three types of erythropoiesis can usually be detected, hypochromic, megaloblastic and normoblastic, the latter, however, usually predominating.

11. The morphology of the peripheral blood is described. Hypochromic cells are concentrated in the central parts of the smear whereas orthochromic

macrocytes are collected at the tail. This is usually so characteristic that a diagnosis of dimorphic anaemia can be made at once in most cases.

12. These views must not be construed to deny the fact that in well-fed persons malaria produces a pure haemolytic anaemia and hookworms a pure post-haemorrhagic iron-deficiency anaemia, neither of which comes under the category of dimorphic anaemia.

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STUDIES ON HEPATO-LIENAL CIRRHOSIS IN EASTERN MEDITERRANEAN COUNTRIES.

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Diseases of the hepato-lienal system are of frequent occurrence in tropical and sub-tropical countries, and they constitute a high percentage of hospital admissions. In the majority of the cases the cause is found to be some chronic parasitic blood infection such as malaria or kala-azar, but there is a group of cases, characterized clinically by anaemia and splenomegaly, which obviously are not connected with a specific protozoal blood infection. Before entering into a discussion of the pathology and probable etiology of this condition we shall review briefly its clinical manifestations as seen in Palestine.

The disease starts in early adult life though it may at times be seen in young children. The earlier manifestations are a moderate enlargement of the liver accompanied by periods of irregular fever of varying severity. The fever may sometimes be typhoidal in character lasting several weeks or there may be short periods of remittent fever followed by long afebrile periods. Chills are not a usual feature. The spleen may be just palpable or not at all while the liver may be felt about three to four fingers under the costal margin. There is no marked tenderness of the liver and the only complaint at this stage is the fever. The blood picture is not characteristic. There may be a slight leukocytosis at the height of the fever, anaemia is slight and the thrombocytes are normal. Blood sedimentation is either normal or slightly increased (15 to 20 mm. in 1 hour). The diagnosis at this stage is not easy, and the patients are usually diagnosed as hepatitis of unknown origin. Emetin treatment is of no avail in reducing the fever or the size of the liver.

As the disease progresses a second stage is reached, characterized by a gradual enlargement of the spleen which may attain enormous proportions while the liver remains stationary. The attention of the treating physician is thus focussed on the spleen which with the rapidly progressing anaemia and leukopenia dominates the picture. The febrile periods are now shorter and

* We beg to express our thanks to Colonel G. W. HERON, Director of Medical Services, Palestine Government, and to Dr. G. STUART, Deputy Director (Laboratories) for permission to publish this article.

far distant, the temperature seldom reaching any considerable height. The patients seek treatment on account of general debility, pains in the abdomen, either diffused or localized over the spleen region (as so vividly testified by the innumerable cautery marks), cough and at times a slight haemoptysis. Haematemesis is not frequent at this stage. Clinical examination reveals a slight enlargement of the heart and haemic murmurs and at times, definite signs of organic valvular involvement. The skin is extremely pale, and pellagroid rashes over the dorsum of the hands and feet may be present. These rashes respond to nicotinic acid treatment. The blood shows a leukopenia, hypochromic anaemia and thrombocytopenia. The total white cell count ranges between 2,000 and 5,000 leukocytes, but during the febrile periods a slight leukocytosis may be present. The red cell count varies between 1 and 3 millions, while the haemoglobin may be as low as 20 per cent., the usual finding being 35 to 45 per cent. A slight relative lymphocytosis is the rule and an eosinophilia, up to 12 per cent., may be found in the presence of intestinal parasites. The thrombocytes vary between 40 and 100 thousand per c.mm. The blood sedimentation is very rapid (60 to 110 mm. in 1 hour by the Westergreen method). The urine contains traces of albumin and, at times, a few erythrocytes, leukocytes and granular casts.

After a period of several years a third stage of the disease is reached when the mechanical effects of the cirrhosis become paramount. Ascites makes its appearance and may reach huge proportions requiring frequent tappings. The liver may still be palpable or it may have shrunk under the rib margin. The spleen is felt to be of enormous size even through a fluid-filled abdomen. Haematemesis may now become a prominent symptom. Jaundice is rare but it may form a part of the terminal picture. This stage may last from 1 to 3 years.

The disease is very debilitating and finally causes the death of the patient in early middle age. The final events are the same as in Laennec's cirrhosis but more frequently the patient succumbs to some intercurrent infection. The low resistance of these patients to infection is striking, and they show a complete anergy towards the injection of bacterial vaccines. The pneumococcus is a specially dangerous invader as pneumonia in a case of cirrhosis was practically always fatal in the pre-sulphapyridine era. On the other hand, it is equally striking that we never met such a patient suffering from a fever of the enteric group.

Sometimes the fully developed picture of cirrhosis with ascites is seen in children at the age of 12 to 14 years. In these cases the disease starts earlier and runs a much shorter course. On the other hand, the disease is seldom seen in people over 50 years of age.

There is no difference in the sex distribution of the disease, but as far as social incidence is concerned the disease is almost exclusively found among the poor rural Arab population.

Pathology.

The clinical picture described above hardly differs from similar conditions described in other countries around the Mediterranean basin. Thus, Griesinger, (1856,) described the condition in Egypt under the name of splenic anaemia. In 1894, Banti described a similar condition in Italy and differentiated it from Laennec's cirrhosis. Banti considered the condition to be a primary splenic fibrosis with secondary liver changes. Ferguson and Day (1909) published an exhaustive study of the Egyptian type and called it Egyptian splenomegaly. Yenikomshian (1934) has described a similar condition in Syria under the name of non-alcoholic cirrhosis. If we accept the view of the earlier authors who ascribe the condition to a primary splenic derangement, we are unable to identify the disease as seen in Palestine with what is known as Banti's disease, in spite of the similarity of the clinical picture. On the other hand the consensus of opinion today tends to regard Banti's disease as a primary affection of the liver with consequent splenomegaly. Thus, Fox (1933) in a study of twenty-three cases, found that the liver damage occurred early; and McMichael (1934, 1935), reviewing forty-five cases, concluded that the splenomegaly was due to venous congestion following cirrhotic changes in the liver. Lately, W. P. Thompson (1940) accumulated evidence to show that in Banti's disease the splenomegaly is secondary to an impairment of the blood flow due, most frequently, to intrahepatic obstruction by periportal fibrosis and occasionally by mechanical extrahepatic obstruction (compression or strangulation). The fundamental difference between Laennec's cirrhosis and Banti's disease appears to be that in the former the pathogenic factor affects simultaneously the liver cells and the perilobular tissue (Siegenbeek van Heukelom, 1896; E. Kaufmann, 1931), while in the latter the *fons et origo morbi* is the increased fibrosis of the periportal tissue alone which may exist for a considerable time without appreciable damage to the lobular structure. And that is exactly the picture described by Ali Pasha Ibrahim (1928) in a number of cases of Egyptian splenomegaly. The same picture was also found in five of our cases where a postmortem examination could be carried out. We are therefore inclined to see in Banti's disease, in Egyptian splenomegaly, and in the Syrian non-alcoholic cirrhosis one morbid entity brought about by the same primary pathological process, namely, periportal fibrosis.

This process seems to exist quite frequently in a latent stage without clinical symptoms. Ferguson found cirrhotic changes in the liver in 10 per cent. of his autopsies. Both alcohol and syphilis were excluded. Only about one-half of these cases had died as a direct result of the cirrhosis while in the other half the cirrhosis was found incidentally. A very early case of periportal fibrosis in a man 25 years old, who died of septicaemic plague after an illness of 2 days was seen by us. Postmortem examination showed a moderate enlargement of the liver and spleen; an intact lobular structure of the liver and a periportal fibrosis, as well as a slightly increased bile duct formation. These changes

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cannot, of course, be attributed to a septicaemia of 2 days' duration. The spleen showed a marked engorgement of the sinuses but no definite fibrosis. In four other cases with considerably enlarged livers and spleens, who died from a short lasting pneumonia with pneumococcal septicaemia, the histological picture was much the same as in the case mentioned above: A moderate perilobular fibrosis of the liver with a more or less marked round cell infiltration in the perilobular spaces. The lobular tissue was essentially normal with the exception of a few scanty small areas of degenerated liver cells which might be attributed to the acute infection. In two cases there were multiple acute abscesses dispersed over the whole liver tissue without any special localisation and showing the presence of pneumococci in the Gram stained slides. The spleens showed a slight thickening of the trabeculae, but did not otherwise differ from spleens of acute infections. There was some blood pigment in the livers and spleens (as mentioned also by ALI PASHA IBRAHIM in his Egyptian cases) but no malarial parasites were found. We had no occasion to examine a case in the advanced stages of the disease but observers in Egypt report that a typical advanced portal cirrhosis of the liver with considerable fibrosis of the spleen is the picture seen in these cases.

ETIOLOGY.

Theoretically it is quite possible that different agents may lead to the same pathological reactions so that the same process may have a multiplicity of causes in different countries. It is, however, striking that the condition under review is practically unknown in England (McNEE, 1931) and Northern Europe (EPPINGER, 1920), while it is more or less frequent in the Mediterranean countries and in the Orient. YENIKOMSHIAN believes that in Syria, a combination of malaria and amoebic dysentery may be the cause. Kala-azar has often been blamed. Repeated examinations of thick films from the peripheral blood during the febrile periods, as well as spleen punctures carried out by us, were invariably negative for plasmodium and leishmania. The formaldehyde test in the serum was equally negative in all cases. Alcohol has been definitely excluded in our cases as well as in Syria by YENIKOMSHIAN, and in Egypt by various authors. The Wassermann reaction in our cases was always negative.

DAY attributed the disease in Egypt to the deposit of eggs of *Schistosoma mansoni* in the liver. His views were not generally accepted (IBRAHIM *loc. cit.*; SORROUR; 1928). At any rate, DAY's theory is not applicable to Syria and Palestine as schistosomiasis does not exist in Syria (YENIKOMSHIAN) and in Palestine, though urinary schistosomiasis is found in certain areas of the southern district its intestinal form is rare, while cirrhosis is more common and evenly distributed over the eastern Mediterranean shores. However, in order to exclude schistosomiasis, we have carried out intracutaneous tests with a *Schistosoma* antigen* in twelve cases and stool examinations for intestinal

* We are indebted to Professor KHALIL BEY, University of Cairo, for putting this antigen at our disposal.

parasites in all our cases. The skin tests were always negative and no schistosome eggs were found in the stools.

We may therefore exclude schistosomiasis as a direct etiological factor, but, on the other hand, perhaps the most remarkable etiological feature of the disease is its close association with intestinal parasites. MANSON-BAHR (1929) mentions its close association with *Ankylostoma duodenale* in Egypt, while its association with *Schistosoma* was mentioned above. YENIKOMSHIAN noted the common incidence of helminthic infestation in Syria and mentioned in order of frequency *Trichocephalus dispar*, *Taenia saginata*, *Ascaris lumbricoides* and *Oxyuris vermicularis*. In Palestine, out of 2,409 stool examinations carried out in our laboratory during the year 1940, helminthiasis was found in 456 (17·3 per cent.). These examinations were carried out as a matter of routine on patients admitted to the hospital for various complaints. In a series of cases of cirrhosis the findings were much higher: 47 per cent. of these patients having eggs of various worms, while 11·8 per cent. had *Entamoeba histolytica* (cysts or vegetative forms). In some cases up to five or six different kinds of parasites were found. One such case had no less than the following combination: *Giardia*, *E. histolytica*, *Trichocephalus*, *Strongyloides*, *Ankylostoma* and *Ascaris*. Yet there was no numerical preponderance of any one of these parasites, so that none of them could be considered the direct cause of the disease. We were inclined, therefore, to see in parasitic infestation a predisposing rather than a causative factor.

The whole clinical picture of the disease points to some infection as the causative factor. In view of the fact that the supposed infection is never fatal by itself but only by sequelae, no highly virulent organism can be expected to be responsible. More likely, an organism of low virulence, invading the body at more or less frequent intervals probably reaching the liver through the minute injuries of the intestinal mucosa caused by parasitic infestation, might in time cause an inflammatory reaction in the liver, followed by fibrosis due to allergic and reparatory processes. According to this theory, the attacks of fever would probably mark the fresh invasions of the supposed micro-organism, and during these periods it might be possible to recover it from the blood stream in some of the cases. We, therefore, performed a great number of blood cultures by inoculating 200 c.c. of glucose broth with 5 to 8 c.c. of the patients' blood. The inoculation was done at the patients' bedside, and utmost care was taken with regard to sterility. The inoculated medium was placed forthwith in the incubator and observed for 10 days. As soon as turbity became visible a loopful of the culture was plated on 5 per cent. rabbit's blood agar.

All examinations carried out during the afebrile and subfebrile periods, and many others during the height of the fever, gave negative results. But in five cases we succeeded, at the time of the fever, in culturing various strains of the same organism, namely, *Staphylococcus pyogenes*, and in two cases the same strain was cultured twice from the same patient on two consecutive days. In all cases, the first culture took from 2 to 7 days to become turbid, presumably

due to the small number of organisms present in the blood of the patients. Subcultures grew luxuriantly in normal time and were easily emulsifiable.

The various strains were all of the same microscopical appearance: Gram positive cocci mainly in pairs, sometimes single or in short chains, rarely in clusters; they all formed acid from glucose, maltose and saccharose, but otherwise they exhibited somewhat varying properties as seen in Tables I and II.

TABLE I

STAPHYLOCOCCI CULTURED FROM THE BLOOD OF PATIENTS WITH HEPATO-LIENAL FIBROSIS.

No. and Name of Patient.	Sex.	Age.	General Remarks.	Agar Colour.	Culture Growth.	Haemo-lysis.	Plasma Coagulation.	Acid formation in	
								Lactose.	Mannite.
1. Saud	M.	34	Isolated twice	White	Viscid	+++	O	+	±
2. Deeb	M.	28	—	White	Viscid	+++	O	+	O
3. Zahra	F.	26	—	Light golden yellow	Butyrous	++	O	O	+
4. Fatmeh	F.	18	Isolated twice	White*	Viscid	+++	O	+	O
5. Fathieh	F.	12	†	(A) Intense golden yellow	Slightly rough	+++	+++	+	+
				(B) White	Viscid	+++	O	+	O

* Many colonies with a yellow centre and white edge.
† In the same culture two different types of colonies were found: (A) very numerous; (B) scanty.

TABLE II.

AGGLUTINATION OF THE STAPHYLOCOCCI FOUND IN CIRRHOSIS WITH AN AGGLUTINATING RABBIT SERUM PREPARED BY INJECTIONS OF STRAIN "SAUD."

Strain.	Titre.	Strain.	Titre.
Saud ...	1/320	Fatmeh ...	1/240
Deeb ...	1/320	Fathieh A	1/160
Zahra ...	1/160	Fathieh B	1/40

We see the whole gamut of *S. pyogenes* variations represented in our findings, from the higher pathogenic plasma-coagulating *aureus* type down to the low grade but not yet wholly saprophytic haemolizing *albus* type. It is perhaps not a mere chance that the former was found in the youngest patient. Serologically, only the first two strains seem to be identical while the others are more or less different. Thus, it did not seem to us of great importance to follow up the question of antigenic structure to a greater detail.

As in most staphylococcal infections the sera of the patients failed to agglutinate their own strains to any appreciable degree and we did not succeed in eliciting a skin reaction by the intracutaneous injection of vaccines prepared from these strains. Recent experiments with phagocytosis tests seem to indicate that by this method a specific reaction may be attained, but our studies in this respect are not yet concluded.

Although the cocci were recovered from the blood stream of the patients, this fact alone would not be a sufficient proof of their etiological role in cirrhosis, as transient bacteriemias of harmless invaders may occur in any emaciated person. We have, therefore, tried to reproduce the disease experimentally in animals and have chosen the rabbit as one rather susceptible to staphylococcus infection. However, not all the strains were suitable. Strain Fathieh A, for instance, was so highly pathogenic for rabbits that 1/50 of a slope agar culture killed a rabbit of 3 kg. in weight within 24 hours. We decided, therefore, to use one of the least pathogenic types and chose Strain Saud, which did not cause any acute disease even if given in high doses. (Though not very virulent, this strain is of a remarkable resistance ; 1/10 of a slope agar culture was injected into an ear vein of a rabbit and the animal was killed 1 month later. The liver and spleen were removed in a sterile manner, cut across and smeared over an agar plate. Very numerous staphylococcal colonies were found the next day in the culture from the liver but only two in the one from the spleen.)

As we suspected the site of entrance to be the intestinal mucosa, we injected a suspension of our cocci in the mesenteric vein of three rabbits. Two other rabbits were injected with the suspension in one of the ear veins. As controls, we injected two rabbits with a suspension of enterococci and two others with a suspension of *B. coli* recovered from rabbit stools, in the mesenteric vein. The control rabbits injected with *B. coli* developed acute purulent peritonitis and septicaemia and died 2 weeks after the injection. All the other rabbits lived and were injected 2 months later with a second dose of the same strain used in the first injection. The first dose injected was 1/10 of a 24 hours slope agar culture and the second dose was 1/5. It would have certainly been nearer to natural conditions had we injected smaller doses at more frequent intervals, but this would have implied a too frequent opening and closing of the peritoneal cavity.

The rabbits injected with enterococci died 1 month after the second injection of chronic septicaemia and adhesive peritonitis, but the animals injected

with staphylococci survived. However, after the second injection, the animals injected by the mesenteric route started to lose weight and developed a moderate anaemia and leukocytosis. The same changes, but to a lesser degree, were observed in the animals injected in the ear vein. (See Table III.) Four months after the second injection all the animals were killed and a postmortem examination was performed.

TABLE III.

Rabbit Number.	Route of Injection.	Weight in grammes.		Erythrocytes (in millions)		Leukocytes	
		Before injection.	6 months later.	Before injection.	6 months later.	Before injection.	6 months later.
1	Mesenteric	1,940	1,800	4·5	3·9	7,900	11,000
2	"	3,290	2,250	5·3	3·3	6,400	12,000
3	"	2,285	2,100	5·2	4·5	8,000	15,000
4	Ear	2,440	2,380	4·8	4·2	7,800	10,000
5	..	2,450	2,560	4·5	3·9	7,500	9,000

POSTMORTEM FINDINGS.

Lungs.—There was a chronic interstitial pneumonia in all animals, irrespective of the route of injection. The alveoli were free but occasionally they were compressed by a round cell infiltration of the septa. The same changes were found in the animals injected with enterococci as well as with staphylococci.

Spleen.—Here again the changes were the same in all animals whether injected with enterococci or staphylococci and irrespective of the route of injection. The organ was somewhat smaller than normal, varying between 0·013 and 0·03 per cent. of the body weight of the animal (normal : 0·05 per cent. in our local rabbit). The Malpighian bodies were reduced in number, the sinuses engorged and the trabeculae thickened in some places.

Liver.—Macroscopically the liver was normal in size and appearance with the exception of Rabbit 2, which, incidentally, suffered the most marked loss of weight and anaemia. In this animal, the liver presented a hobnailed surface and a fibrotic consistency on cutting. Microscopically, pathological changes were found in all the animals, but with a fundamental difference between the animals injected with enterococci and those injected with staphylococci. The former infection resulted in multiple milliary round infiltrations scattered indiscriminately over the whole of the liver substance, in other words these minute abscesses were found both in the intra- as well as in the peri-lobular tissue (Fig. I). The staphylococcal infection on the other hand, resulted in a strictly perilobular infiltration without any considerable alteration of the intralobular structure (Fig. II) and only very scanty small islets of vacuolized liver cells without any infiltration within the lobule were found.

It is remarkable that in principle all animals infected with staphylococci showed the same changes, regardless of the route of injection, the changes in the rabbits injected by the mesenteric route being slightly more intense than in those injected peripherally. In Rabbit 2, the infiltration was widely replaced by connective tissue and there was a certain amount of new formation of bile ducts.

FIG. I.—Irregular disseminated abscesses in the liver due to enterococci. × 20.

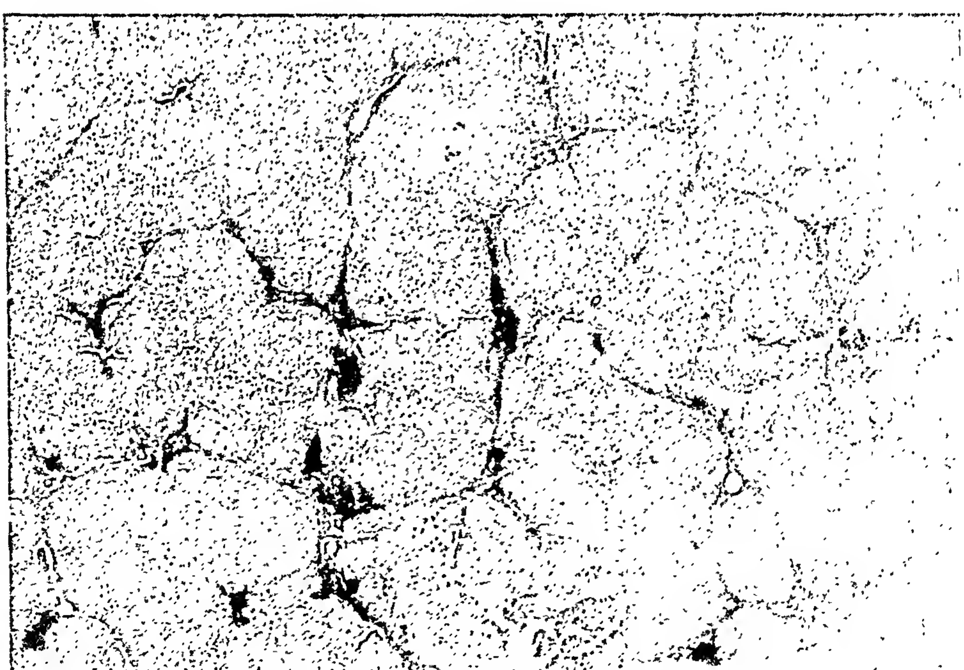

FIG. II.—Perilobular infiltration of the liver due to staphylococci. × 20.

To face page 184]

that the staphylococci are endowed with a certain degree of resistance sufficient to withstand the cellular defence of the connective tissue but not of the reticulo-endothelial system.

But then, is this staphylococcus the specific causative agent of the disease or is it just one of a group of infective organisms capable of producing similar changes? Barring the acute superinfection with pneumococci and *B. pestis*, on only two occasions we were able to culture from the blood stream organisms which, in view of their comparatively low virulence, could be suspected to be of etiological importance, namely, enterococci. In both cases the patients were children (7 and 12 years old respectively). In one instance the enterococci were found alone while in the other case a mixed culture of enterococci and staphylococci were found. (Case 5, Fathieh.) Yet the more frequent finding of staphylococci and the completely different histological picture obtained in experimental infection of rabbits with enterococci (Fig. I) makes us doubt whether we should attribute to the enterococcus a more important role than that of an occasional secondary invader. Findings like those of FERGUSON, who observed irregularly disseminated minute abscesses in the liver of some of his cases of Egyptian splenomegaly, are probably due to such a transitory superinfection. Still, it is conceivable that organisms of the same degree of low virulence and moderate resistance may bring about the same pathological changes. The disease may be compared to endocarditis lenta where usually members of the *Streptococcus viridans* group are found to be the cause but where occasionally other organisms may produce similar pictures.

The similarity of the disease as seen in Palestine to the condition seen in other countries around the Mediterranean, the frequency of intestinal parasitic infestation in all these countries, the identical pathological findings in all these cases, etc., tend to point to all these conditions being one and the same disease. Further studies are required in order to ascertain if in all countries the same agencies are at work. On the other hand, the different age incidence, the different strata of people affected in England and Northern Europe, differentiates this disease from the true Laennec's cirrhosis. In spite of the fact that pathologically the advanced stages of Laennec's cirrhosis are identical with the Mediterranean cirrhosis, we are unable to understand why the spleen should play such a prominent role in the clinical picture of the Mediterranean type. There are probably several factors involved, but we can point to at least two of them (*a*) the age incidence and (*b*) the rate of progress. As far as the age incidence is concerned the Mediterranean cirrhosis starts early when the spleen capsule is still rather elastic, while the Laennec's cirrhosis starts after middle age. In Hanot's cirrhosis, which is also a disease of the younger generation, the spleen is much more enlarged than in Laennec's. Considering the rate of progress of the disease, Laennec's cirrhosis differs from the Mediterranean type by its much shorter duration and therefore more rapid progress. It may be assumed that the combination of

an early onset and a slow progress results in the extreme enlargement of the spleen.

From the study of the incidence of the disease one fact calls for an explanation. Though parasitic infestation is common to all the strata of the population, hepato-lienal fibrosis is found mainly amongst the poorer rural classes. It becomes evident, therefore, that still other factors are at work. LEESMITH reported two of his patients having xerophthalmia. Our own cases often showed signs of vitamin deficiencies in the form of pellagroid rashes. YENIKOMSHIAN, reviewing the diets of these patients, concluded that it was poor in vitamin A and in proteins.* It seems, therefore, safe to assume that nutritional factors play a certain role in the development of the disease.

We are, perhaps, now in a position to state on broad lines the theory of the causation of hepato-lienal fibrosis in this country. In underfed young individuals living under rather insanitary conditions in which intestinal parasitic infestation is rife, an abnormal intestinal flora develops with a preponderance—as is well known—of Gram positive cocci. Some of these, for which the natural defences of the body are less adapted, gain access to the submucous tissue through the small multiple injuries caused by the parasites and reach the intrahepatic portal venules, where, thanks to their low virulence, they cause a mild inflammation which may in time eliminate them. But as new invaders find their way to the portal system the process is kept going and even intensified by the gradually developing local allergy. If the balance between staphylococcus and host be shifted in favour of the former, coccal invasion of the general circulation occurs. Such a shift may be provoked or accompanied by a secondary invader. (See Case 5, p. 182.) These invasions are more violent and frequent in the early stages, but as time goes on the local inflammation is superseded by connective tissue proliferation, which in turn interferes with the blood flow in the portal system causing stasis in the spleen. As repeated bacteriemias are added to the stasis the spleen reacts with a hyperplasia of the pulp and eventually with fibrosis. Such bacteriemias are probably indispensable in the causation of splenomegaly. The blood changes may be due to splenic hyperfunction and in part to the haemolysin and leukocidin of the staphylococcus.

Epitomizing our views on the pathogenesis of this disease we should say that the intestinal parasites open the door, the nutritional deficiencies pave the way and the staphylococcus finishes the work.

SUMMARY.

1. Various strains of *Staphylococcus pyogenes* were cultured from the blood of patients suffering from Mediterranean hepato-lienal fibrosis.

* In this connection, the findings of ELMAN and HEIFETZ (1941) are interesting. These investigators observed degenerative changes in the livers of dogs and a decrease of the albumin fraction of plasma proteins after prolonged protein-free diets.

2. Intravenous injections of one of these strains caused, in rabbits, pathological changes in the liver similar to the changes found in human hepato-lienal fibrosis.

3. The close association of the disease with intestinal parasitic infestation and nutritional deficiencies is noted.

4. It is supposed that the staphylococcus gains entrance to the blood stream through the minute injuries of the intestinal mucosa caused by the parasites and the development of the disease is aided by the nutritional deficiencies.

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RELAPSING FEVER IN ABYSSINIA*

BY

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The appearance of the excellent article by Lieut. A. D. CHARTERS† under this heading tempts me to add the following notes from data prepared by me at the time, though viewing the disease from a somewhat different aspect.

As medical officer to the battalion of West African troops which may be said to have supplied Lieut. A. D. CHARTERS with his soldier patients, my energies were directed more to the preventive side, but all men were seen by me at routine sick parade where a provisional diagnosis was made before they were passed on to the field ambulance for treatment. The errors of this provisional diagnosis will be discussed later.

During the period from 27th June, 1941, to 12th October, 1941, twenty-eight bacteriologically confirmed cases of relapsing fever occurred in this

* Published by permission of the War Office.

† CHARTERS, A. D. (1942). Relapsing fever in Abyssinia. *Trans. R. Soc. trop. Med. Hyg.*, 35, 271.

battalion, and our strength was nearer to 700 than to the figure of 1,000 which CHARTERS mentions. There were a further twelve cases which, on looking back, were almost certainly relapsing fever though we did not diagnose them at the time. The louse vector was confirmed.

The battalion reached Soddu on 7th June after a long march through sparsely populated country; as there was at that time all too much water, and our troops are naturally cleanly, I think it safe to assume that there was then little or no louse infestation. The first confirmed case was admitted on 28th June, diagnosed by me as migraine, and finally diagnosed correctly by CHARTERS on 2nd July soon after his arrival. The simultaneous diagnosis of relapsing fever in soldiers from other units, with two deaths, gave rise to the rapid introduction of such preventive measures as we could devise. These consisted in the delousing of all troops and their clothing at least once a week, together with frequent inspections of all troops at which a very high percentage were found to be louse-infested.

Our method of disinfestation was somewhat elementary, consisting of a 44-gallon drum containing about 4 gallons of water: clothes, blankets, etc., were placed inside upon a wooden stand. The top was then replaced, the edges padded with cotton wool, and the largest stone we could find placed on top. By lighting a fire underneath a very reasonable pressure of steam could be raised, sufficient at times to make the barrels bulge outwards ominously. The troops meanwhile washed their bodies with a strong carbolic solution.

By the time that the other units left Soddu on 7th July these measures were in satisfactory working order; unfortunately, however, closer contacts were now occurring between our troops and the local Abyssinians, especially the younger women who, as Lieut. CHARTERS says, were often heavily infested with lice.

The next confirmed case occurred on 21st July, and two more confirmed cases on 25th July, but it was during this period that nine cases occurred which in retrospect appear as probable relapsing fever cases, and these include, I think, the four which CHARTERS considers as abortive cases with negative blood slides, and the three cases of infective hepatitis in which he considers relapsing fever as a possible predisposing factor.

One further case occurred on 30th July and another on 31st July. On 2nd August a lance-corporal complained of headache, which I considered malarial, and he was fit for duty again on 4th August without ever going to hospital. On 7th August the same man appeared with an obvious relapsing fever and was admitted and blood films were positive for spirochaetes; he was almost certainly a case in which the initial fever was unrecognized.

On 3rd August the fatal case to which CHARTERS refers occurred with death after 2 days. Further cases occurred on 4th August, 5th August, and two more on 14th August, although by this time two of our companies

had left Soddu, leaving about 400 troops only. One of these men died later in Addis Ababa.

On 18th August CHARTERS himself moved from Soddu with his Company of the Field Ambulance, and a further case was put into his ambulance as he was about to leave. This man subsequently died in Addis Ababa.

The last case occurred in Soddu on 19th August, and as all ambulances had already left and he had to travel for 3 days in the front of a lorry his prospects did not appear too good. He received N.A.B. 0·6 gramme on the 19th, a further 0·6 gramme on the 23rd, and though spirochaetes were still present in his blood on admission 24th August, I am glad to say he made a good recovery.

This completed what I may call Phase I of our epidemic, the cases occurring at Soddu which, as CHARTERS says, lies at a height of 7,500 feet, having a cold climate with a heavy rainfall. Of twelve cases occurring there, and receiving their initial treatment there, only one died, and one more died after being transferred to Addis Ababa. Of two further cases occurring there, and treated during a move, one died.

An analysis of the diagnoses made in the unit M.I. Room shows that the cases were diagnosed as malaria in five cases, correctly as relapsing fever in four cases, and once each as migraine, diarrhoea, infective hepatitis, pneumonia and schistosomiasis.

Of the eleven further cases which were never confirmed bacteriologically malaria was diagnosed at the M.I. Room in six cases, in two of these the field ambulance agreed and in four cases I never heard their diagnosis; relapsing fever was diagnosed in three cases, of which two were diagnosed as infective hepatitis and one as malaria subsequently; migraine and infective hepatitis were each diagnosed once, the former being returned as malaria, whilst I never heard of the latter's final diagnosis.

These figures are summarized below:—

TABLE I.

BACTERIOLOGICALLY CONFIRMED CASES.

M.I. Room Diagnosis.	Number of Cases.
Malaria	5
Relapsing fever ...	4
Migraine	1
Diarrhoea	1
Infective hepatitis ...	1
Pneumonia	1
Schistosomiasis ...	1

Total number of cases 14; Deaths occurring in Soddu 1; Deaths occurring after evacuation to Addis Ababa 2.

TABLE II.

PRESUMED CASES: DIAGNOSIS NOT CONFIRMED BACTERIOLOGICALLY*.

M.I. Room Diagnosis.	Field Ambulance Diagnosis.	Number of Cases.
Malaria	Malaria	2
"	Not known*	4
Relapsing fever ...	Infective hepatitis	2
" " ...	Malaria	1
Migraine	"	1
Infective hepatitis ...	Not known*	1

Total number of cases 11; Deaths nil.

* *Note*—It is of course possible that spirochaetes were found in some of these cases, but their subsequent clinical notes never reached me.

It was of some interest that the last case mentioned above had suffered from a penile ulcer 2 months before his attack of relapsing fever and had received his fourth injection of N.A.B. 0·45 gramme only 14 days before the appearance of his symptoms. As we had no laboratory facilities we never knew whether the ulcer was syphilitic or not.

One Abyssinian woman was treated at this time as she developed bacteriologically confirmed relapsing fever in a room adjoining our B.N.C.O.'s mess. Another Abyssinian woman who was sharing the same room, and was at that time acting as interpreter for both CHARTERS and myself, asked us whether she could have a prophylactic inoculation. After discussion we decided against this, and I am glad to say she did not develop the disease, but it would be interesting to know whether any form of drug prophylaxis is possible.

Phase II of the epidemic now occurred between the 24th August and 10th September whilst three companies, approximately 400 men, were in Addis Ababa. Addis lies at a height of 9,000 feet, otherwise both the temperature and the rain were similar to Soddu. Six cases, bacteriologically confirmed, occurred with the appalling figure of five deaths. As two of our Soddu cases had died after reaching Addis, this gave a total of seven deaths.

Of the six cases, three were admitted directly to hospital by the Italian medical officers then taking sick parade for our outlying companies, the remaining three were all recognized as relapsing fever before their admission. One further probable case occurred, with recovery, but I never saw his bacteriological reports after admission.

As a different field ambulance was receiving these patients, enquiries were made and revealed the following difference in treatment technique. Those patients who showed a deep jaundice on admission were not given intravenous N.A.B. as it was considered that the liver involvement must already be too

great to allow of the further damage which arsenicals might produce. Five cases were thus treated with glucose salines and rest; of these four died. One case occurring in Addis did, however, receive N.A.B. 0·6 gramme and yet died, whilst the two cases evacuated from Soddu had both received two injections each of 0·6 gramme, and we eventually came to the conclusion that the higher altitude had a very definite effect in raising the mortality rate. Whether this would be true generally, or whether it was only on our Gold Coast soldiers, used as they are to low altitudes only, that this factor operated we were unable to judge.

Phase III of the epidemic occurred at Giggiga, at the even lower altitude of 2,000 feet. Between 12th August and 3rd September seven bacteriologically confirmed cases occurred, without any deaths. These cases were all receiving N.A.B. 0·6 gramme as a routine, however, so that there was again no control of the effects of altitude alone. Two of these cases were diagnosed as simple constipation on their first attendance, and only admitted the following day, one was admitted as bronchitis and one as pneumonia. These errors in diagnosis bring out well one of the points which we took to be in favour of the theory that altitude increased the severity of the disease, namely, that all the cases seen in Addis showed marked jaundice at the beginning, whilst at both Soddu and Giggiga jaundice was not an early feature, though it did occur later in every case.

The final case in the battalion occurred at Mandera, in British Somaliland, at an even lower altitude of 800 feet, and despite intravenous N.A.B. 0·6 gramme ended fatally. An analysis of the nine fatal cases shows that in all but three, death occurred on the second day after admission (Table III).

TABLE III.

ANALYSIS OF THE NINE FATAL CASES.

Case Number.	Number of Days after admission that Death occurred.	Whether Patient received N.A.B.	Place reporting Sick.
1	2	Yes	Soddu.
2	19	,,	,, Died 5 days after reaching Addis
3	2	,,	Soddu. Died 1 day after reaching Addis
4	9	No	Addis
5	2	,,	,,
6	2	,,	,,
7	5	Yes	,,
8	2	No	,,
9	2	Yes	Mandera

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4	9	No	Addis
5	2	„	„
6	2	„	„
7	5	Yes	„
8	2	No	„
9	2	Yes	Mandera

SUMMARY.

1. Twenty-eight bacteriologically confirmed cases of relapsing fever are reviewed occurring in Gold Coast soldiers in Abyssinia.

2. There were nine fatal cases, six of them occurring on the second day after admission.

3. It is suggested that greater altitude tends to a higher mortality.

4. It is suggested that the treatment of choice is to give intravenous arsenicals to all cases however jaundiced.

5. Twelve other probable cases are mentioned, though spirochaetes were never found in blood films.

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THE EFFICIENCY OF HEADGEAR AS INSULATION AGAINST RADIATION.

BY

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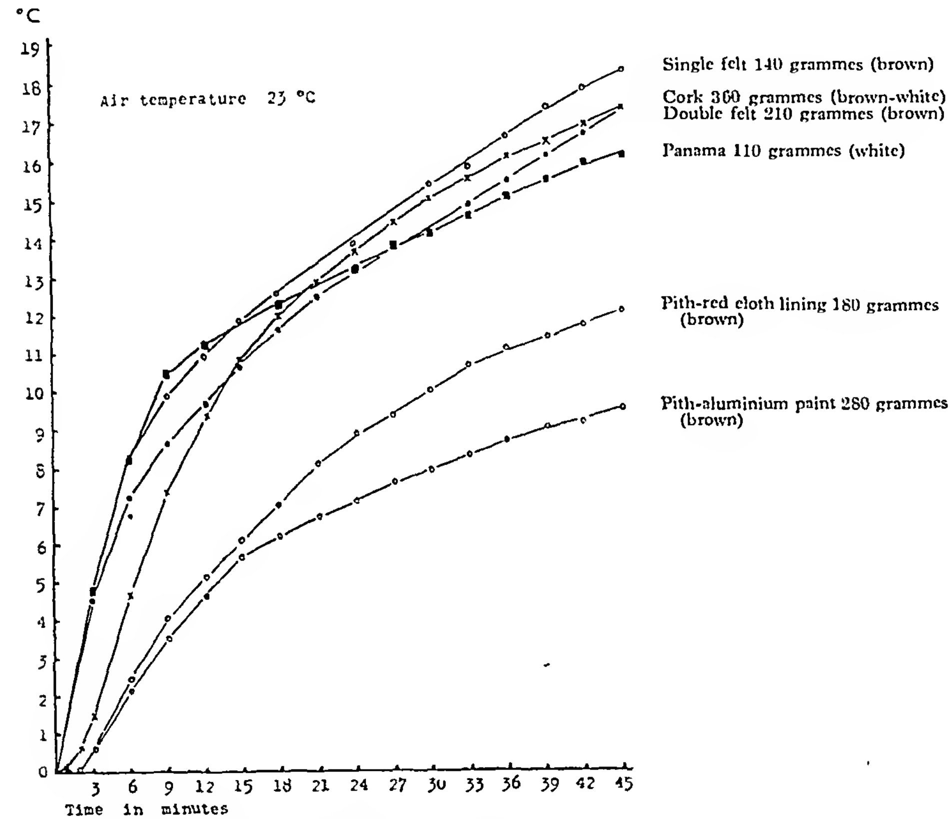
Europeans in the tropics wear many kinds of headgear and apparently give little thought to their efficiency as insulators against the sun's radiation. CORSON* tested, by means of clinical and other thermometers, the resistance of several types of hat to full sunshine (generally in the presence of a strong breeze). He found that a Panama was more efficient than various types of felt hats while a lady's pith helmet and a man's polo hat, both white, were more efficient than the Panama. I have come to similar conclusions by a somewhat different method.

The experiment, which was conducted in the relatively still air of a laboratory, was designed to measure the rise in temperature inside headgear exposed to a standard source of radiation at a fixed distance from the crown of each hat. A 500 watt lamp, in a reflector, was suspended vertically above, and 28 cm. from the crown of the hat under test. As measured by a Gorezynsky solarimeter (N.P.L. tested) the radiation falling vertically on the upper surface of the hat was equivalent to 0·4525 gramme calories/cm.2/min., or roughly one quarter of the vertical component of solar radiation at midday in Amani (alt. 3,000 ft., lat. 5° S.) during the same period (October-November). The hat was supported on an artificial " head " composed of kapok-stuffed cloth roughly of the shape of the human head. On top of the " head " at a point corresponding to the upper parietal region in man, a copper-constantin thermocouple was fixed against the upper surface of the cloth. The hats when being tested were so placed that each was filled by the " head " to the same extent as in normal use. The thermocouple was connected to a cold junction of similar type placed in a thermos flask filled with water at air temperature and both connected to a galvanometer. As air temperature did not vary by 1° C. during the time of each experiment the deflection of the galvanometer is a measure of the difference between air temperature and the temperature inside the hat. The apparatus was calibrated by placing the " head " thermocouple in water at different temperatures and measuring galvanometer deflection per degree Centigrade. At least two different hats of each type were tested. The deflection of the galvanometer when the hat was receiving no radiation from the lamp was then read and taken as zero value. In practice this is close to open-circuit zero. The lamp was then switched on and readings of the galvanometer taken every minute for the first 9 minutes and thereafter every 3 minutes up till 45 minutes after the start of the experiment. Galvanometer readings

* CORSON, J. F. (1926.) *J. trop. Med. Hyg.*, 29, 2.

were converted into their temperature equivalents and for convenience the results have been expressed as a graph in which rise of temperature (° C. above air temperature) has been plotted against the time of exposure in minutes. Average values have been given for all the types.



It can be seen from the above graph that the most efficient hats were the pith and of these the heaviest (having an aluminium paint lining) was the best. They gained heat slowly and the curves show marked flattening at the end of 45 minutes, indicating that temperature inside the hats was approaching stability. Of the remaining types the cork helmets were the most efficient (white and khaki equally so) for short exposures up to 10 minutes' duration but not so efficient as the Panama hats at the end of 45 minutes. The curves of both show marked signs of flattening at the end of this period when compared with the curves for the single- and double-felts. The double-felt hats gained heat more slowly than either the Panama or the single-felts over the first 15 minutes, but the slope of the curve, which is parallel to that of the single-felts after 12 minutes' exposure, shows that it is probably much less efficient than the cork helmet at exposures longer than 45 minutes. The single-felt hats were the least efficient of all types tested.

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COMMUNICATIONS.

ANURIA.

WITH SPECIAL REFERENCE TO RENAL FAILURE IN BLACKWATER FEVER, INCOMPATIBLE TRANSFUSIONS, AND CRUSH INJURIES.

BY

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CONTENTS.

Introduction.

The purpose of this paper is to examine the present status of the problem of anuria and oliguria occurring in such syndromes as blackwater fever, favism, incompatible transfusions and crush injuries and to suggest that the renal abnormalities occurring in all these diseases have a similar basis. The hypothesis of Baker and Dodds (1925) and Baker (1937) that the anuria occurring in the haemoglobinurias of incompatible transfusions, blackwater fever, and in rabbits injected with haemoglobin solutions, is the result of mechanical blockage of the renal tubules with haemoglobiniferous products, especially when the urine is acid, is insufficient to account for all the facts, and in the light of recent research seems an improbable explanation. Their view that oliguria and anuria can be prevented by the simple process of " alkalizing " the urine is not borne out in the majority of cases, and on physiological grounds seems unlikely.

Earlier Observations.

Other workers had emphasized the occurrence of anuria after acute intravascular haemolyses. Plehn (1903) who investigated the problem of anuria in blackwater fever regarded nervous inhibitions as the chief factor in bringing about urinary suppressions in this disease. We shall return to this aspect of the question when considering the later investigations of Mason and Mann (1931) and Hesse and Filatov (1933).

Ponfick (1883), Werner (1907), and de Haan (1905) demonstrated that the kidneys of patients dying from blackwater fever contained granular material in the lumen of the tubules and attributed the urinary suppression to mechanical blockage of the tubules. None of these authors, however, suggested that the presence of granular material or the blockage of the tubules might have been the result of diminished glomerular filtration and inadequate " flushing " resulting therefrom. Barratt and Yorke (1909) carried out a comprehensive investigation of the kidneys in blackwater fever, later Yorke and Nauss (1911) working with rabbits produced haemoglobinuria by injection of solutions of haemoglobin containing 1·5-13·0 grammes into the circulation. In a certain proportion of these rabbits anuria developed, and Yorke and Nauss concluded that this anuria was due to occlusion of the lumen of the renal tubules with plugs of granular material derived from haemoglobin. They emphasized, however, that any factor which tended to lower blood pressure and thus decrease glomerular filtration, considerably increased the tendency to blockage of the renal tubules ; and that if blood volume was maintained by feeding the animals on a moist diet, or by saline injections, considerable quantities of haemoglobin could be injected without producing urinary suppression. These authors commented on the extreme dilatation of many of the renal tubules and the obliteration of the lumen of the adjacent tubules, by pressure, in their anuric animals : no doubt a factor of importance in assessing glomerular filtration. They further

made observations on the haemoglobin levels in blood and urine that have been confirmed by recent studies, and an examination of their paper of nearly 30 years ago reveals that many of the views expressed by these authors are in accord with modern theories of kidney function. (LICHTY *et al.*, 1932; HAVILL *et al.*, 1932; MONKÉ and YUILE, 1940.) That nervous mechanisms may also play a part in renal secretions has been shown by HESSE and FILATOV; and THEOBALD and VERNEY (1935) and GRUBER (1933).

THE ALKALIZATION HYPOTHESIS.

BAKER and DODDS, examining the question of renal failure in some conditions involving intravascular haemolysis, suggested that the anuria was due to mechanical blockage of the tubules with precipitated products of haemoglobin from a highly acid urine with sodium chloride content above 1 per cent., and stated that the azotemia and anuria could be avoided by alkaline therapy. Their work was based on the results of experiments with three rabbits, and renal failure was judged by rising blood ureas. McCANCE (1936) has shown that in uncompensated alkalosis the uraemic state is probably due to fall in glomerular filtration. KERPEL-FRONIUS (1936a), and BLACK (1942) and many others have also shown that there is a consistent relationship between dehydration and azotaemia. This hypothesis of alkalization has, however, so dominated the field of treatment in cases of oliguria and anuria of blackwater fever and incompatible transfusions during the past 15 years that it has tended to overshadow modern developments in renal physiology and alternative methods of treatment. In blackwater fever there is no evidence that oliguria and anuria are commoner in patients with acid urine than with alkaline; in fact, anuria develops frequently in patients that have had a small haemolysis and passed alkaline urines, and fails to develop in patients who had a large haemolysis, as shown by blood counts, and who pass consistently acid urines (FOY and KONDI, 1941).

ZIEGLER and BRICE (1937) suggest that phylogenetically the human kidney is adapted for the excretion of an acid urine and classes man on this account nearer to the carnivorous animals than the herbivorous, and states that the human kidney can more easily excrete solids in an acid than alkaline urine, and this is confirmed by DE NAVASQUEZ (1940). BRIDGES and MATTICE (1940), STEELE (1936), LASHMET (1931), have called attention to the undesirable effects of indiscriminate alkali therapy and point out that in such cases there is diminished nitrogen excretion and failure of the kidney to excrete the injected alkalies, thus leading to alkalosis while the urine is still acid. The administration of alkalies has long been recognized as a dangerous procedure in persons with renal impairment and who are azotemic (BERGER and BINGER, 1935; HARDT and RIVERS, 1923; KEITH *et al.*, 1942; HOFF *et al.*, 1941; SCUDDER *et al.*, 1937). It should also be remembered that the efficacy of acidifying and alkalizing drugs is conditioned by the amount of water that is simultaneously demanding excretion. In this connection RIGDON and CARDWELL (1942) have shown that the

necrosis that occurs in the renal tubules after intravenous injection of hypertonic sucrose only occurs in dehydrated animals, and can be prevented by maintaining an adequate fluid intake.

Nicol (1940) has further stated that in alkalosis dehydration is the most important factor leading to the development of renal damage, and that only in dehydrated patients did albumin and casts occur in the urine, and concludes that dehydration leading to haemoconcentration, and consequent impairment of renal blood supply is the chief cause of renal tubular damage in alkalotic conditions; that in spite of alkalaemia the reaction of the urine may remain acid, and that the damaged renal tubules fail in their function of secreting alkalies, and thereby aggravate the alkalaemia.

As alternative therapy, Wakeman *et al.* (1932) suggest blood transfusion and intravenous saline glucose. These authors observed instances of alkaline therapy leading to alkalosis, and that in salt-depleted patients secretion of an acid urine continued in spite of blood alkalosis. In their opinion, the maintenance of adequate urine volume would be likely to offset the effects ascribed to an acid urine, thus in their view also urine volume is more important than reaction.

Where vomiting is present this will lead to serious salt depletion and electrolyte disturbance. Diarrhoea by causing water loss will likewise upset electrolyte balance.

These symptoms occur in most intravascular haemolyses and are themselves capable of producing renal dysfunction that will not be associated with mechanical blockage. Wakeman *et al.* have commented on these factors in blackwater fever and realise that they produce conditions unfavourable to the secretion of urine. In such conditions sodium chloride medication is desirable, and an adequate fluid intake must be maintained.

De Gowin *et al.* (1934, 1937, 1938) and de Gowin (1938) have reported renal insufficiency after incompatible transfusion, and in dogs injected with solutions of haemoglobin. They state that free haemoglobin in the plasma is a potent renal toxin, but add that it is impossible to decide whether the vasoconstriction as reported by Mason and Mann; and Hesse and Filatov is more important than blockage and tubular degeneration in the causation of the azotemia.

De Gowin and his collaborators used two groups of dogs in their experiments; one group passing urine made acid with large doses of ammonium chloride, and a second on a normal diet and passing alkaline urine. The acid dogs sometimes died with azotemia, the renal tubules showing blockage, which they state was the chief cause of the renal failure, although tubular necrosis if severe enough might cause death. They add, however, that other physiological disturbances could not be ruled out in the acid dogs.

Only two of the acid dogs were anuric at the time of death, all the others passing adequate amounts of urine. In some of the acid animals there was

maximum necrosis and little blockage, in others there was extensive blockage but little necrosis, making it appear that blockage and necrosis are two independent phenomena, although producing similar clinical pictures. Acid urine is regarded by DE GOWIN as an important factor in bringing about renal changes, since the alkaline dogs showed neither azotemia nor tubular changes.

Of the seven dogs showing symptoms of gross renal insufficiency, five passed adequate quantities of urine up to the time of death, which would have been impossible had mechanical blockage been effective. The effects of the administration of such large amounts of ammonium chloride would tend to produce acidosis, diuresis and reduction in blood base, the consequences of which on renal function have been discussed above and it is further likely that acid dogs would be rendered more susceptible to the deleterious effects of haemoglobin injections.

ALBRIGHT and BAUER (1929) have shown that the administration of ammonium chloride in nephrosis causes a sweeping out of base from the organism. DE GOWIN *et al.* state in their protocols that the acid dogs vomited and that they failed to retain water given to them. They have not, however, taken these important factors into consideration in their conclusions. It will be pointed out below that electrolyte-acid-base-water balance disturbances are very important factors in the development of extra-renal azotemia, and in controlling glomerular filtration and tubular reabsorption, which will in turn influence dehydration and haemoconcentration. No data are given by DE GOWIN on these aspects of the question. It seems, therefore, that their findings in the acid dogs can be better explained as resulting from electrolyte-acid-base-water balance disturbances leading to dehydration actual and physiological, haemoconcentration, reduction in renal blood flow and glomerular filtration, and these combined with the presence of large amounts of haemoglobin in the tubular filtrate leading to degeneration of the tubular epithelium. It is also likely that with such high doses of ammonium chloride the electrolyte concentration of the urine would in ordinary circumstances be high and since the dogs were vomiting in addition it follows that a still more concentrated urine would be secreted with relatively greater amounts of haemoglobin. A closer scrutiny of the paper by DE GOWIN and BALDRIDGE (1934) hardly justifies their statement that their findings support the conclusions of BAKER and DODDS.

These facts considered together lend support to the view that tubular blockage *per se* may not be the cause of the renal symptoms, and that the blockage is itself determined by antecedent factors, of which diminished glomerular filtration due to whatever causes is an important entity since it will lead to inadequate " flushing " that will facilitate the deposit of material in the lumen of the tubules.

It is suggested, however, that no single explanation can account for the changes in renal function and the anuria that occurs in the intravascular haemolyses, but that they are due to a series of events starting off with the sudden

haemolysis, and leading to the other symptoms that are characteristic of all these conditions. Among other factors which may be involved in reducing renal function we propose to examine the following :—

Effects of Haemoglobin on Renal Metabolism.
Haemoglobinaemia in Connection with Permeability Changes.
Electrolyte-Acid-Base-Water Balances.
Osmotic Pressure in Relation to Filtration and Reabsorption.
Changes in Glomerular Filtration and Tubular Reabsorption.
Urinary Pigments in Relation to Blockage.
Quantitative Relation between Tubular Blockage and Renal Function.
Protein Catabolism in Relation to Azotemia.
Favism.
Sulphonamide Haemoglobinurias.
Crush Injuries.

A. Effects of Haemoglobin on Renal Metabolism.

Whatever toxic substances are assumed to cause the sudden haemolysis may equally well exert a deleterious effect on the glomeruli and tubular cells, or indeed on the kidney in general. Since, however, the injection of large amounts of haemoglobin into animals whose fluid intake is inadequate may produce changes in renal function, it is also possible that haemoglobin may directly or indirectly be a toxic substance.

Mason and Mann found that the injection of stroma free haemoglobin in saline caused an immediate fall in the volume of the kidney, and stoppage of the secretion of urine in dogs with exteriorized ureters. They further state that the injection of 0·2 gramme of haemoglobin in 0·5 c.c. of 0·4 per cent. sodium chloride caused a definite slowing up of the blood flow to the kidneys, a narrowing of the large vessels, and a reduction by 50 per cent. in the number of working glomeruli. They conclude that the renal vessels will tolerate a certain amount of free haemoglobin in the plasma, but that when the amount has reached a certain threshold the renal vessels contract. These authors also found that the injection of cell stroma had no such effect, and assumed that the haemoglobin in solution produced the results noted by them. Hesse and Filatov state that a depressor substance is liberated from broken down erythrocytes, causing an arterial spasm, dilatation of the capillary bed, and a fall in blood pressure. Derangement of renal function may follow as a result of the toxic action of the products liberated by the broken down red cells. Eggleton *et al.* (1940), White (1939) and Winton (1937) consider that variations in the number of working glomeruli in the mammalian kidney do not occur, and that normally variations in glomerular filtration do not affect the volume of the urine. Smith (1937), Hayman and Starr (1925) and Richards and Plant (1922) have produced evidence to show that in the rabbit variation

in the number of functioning glomeruli occurs, is dependent upon renal blood flow and volume, and other things being equal will affect the volume of the filtrate (PETERS, 1935). Severe dehydration may cause variation in the number of functioning glomeruli, such as probably occurs in the frog: it may happen in the mammal, and is more likely in the rabbit than in the dog (WHITE, 1939). BORCHARDT and TROPP (1928) state from their experiments with injecting haemoglobin solution into dogs, that it is the globin moiety of haemoglobin that is toxic and that if "pure haemoglobin" solution is used no toxic effects result. BAKER and DODDS overcame the toxic effects by filtering their haemoglobin before use, but whether this contributed to their more favourable results is difficult to say.

REID (1929) found that the injection of distilled water into dogs caused a marked decrease in renal volume when given intravenously in amounts of 0·5 to 1·0 c.c. per kg. of body weight: red cells lysed with distilled water and made hypertonic with 2·0 per cent. sodium chloride also caused marked decrease in renal volume. REID concluded that the renal volume decrease was due to a substance liberated when red cells are injured. No such effects were produced when serum treated in the same way was injected.

BORDLEY (1931) suggests that in incompatible transfusion, the toxic effects are due to the incompatible blood. In such cases it seems to us that it is not haemoglobin *per se* that causes the toxic reaction, since in paroxysmal haemoglobinuria and blackwater fever very much larger quantities of haemoglobin are liberated into the plasma without causing such profound immediate reactions.

There appear to be three types of reaction to incompatible transfusion:—

(1) A severe collapse with lumbar pain and rigor immediately following the administration of a very small quantity of incompatible blood (10 to 100 c.c.) (Landsteiner Groups.)

(2) A delayed and less severe reaction, accompanied by haemoglobinuria, jaundice and methaemalbuminaemia, following the injection of much larger amounts of blood (500 c.c. or more) associated with some of the lesser known grouping factors (Rh. factors, and other subgroups, WIENER 1941).

(3) Haemolytic reaction occurring after the injection of high titre donors' serum which may result in lysis of the recipient cells, accompanied by an increase in the indirect van den Bergh reaction (AUBERT *et al.*, 1942).

In the case of (1) it is rare for patients to survive the lysis of much more than 100 c.c. of donors' cells and even small amounts may be rapidly fatal (WIENER, 1939). Although only such a small number of red cells are lysed the reaction is immediate and very severe and may be followed by oliguria and azotemia. In the case of (2) and (3) the haemolysis may be much greater, yet the immediate symptoms are not so severe, but haemoglobinuria oliguria, anuria and azotemia may follow.

In blackwater fever the situation seems to resemble that in (2) and (3)

but in addition in blackwater fever there is lysis of both donors' and patients' cells in transfused cases (Foy *et al.*, 1941).

In certain cases of incompatible transfusion methaemalbumin is said to appear in the blood (Fairley, 1941); in such cases it seems likely to us that more than a 100 c.c. of blood would have been lysed, since the injection of 15·0 to 25·0 grammes (180 to 350 mg. per kg. bodyweight) of haemoglobin fails to produce methaemalbuminaemia. We think, therefore, in those cases of incompatible transfusion where there is methaemalbuminaemia the reaction falls into type (2).

It seems to us that the reaction that occurs in type (1) is probably due to causes other than haemoglobin, and that the renal failure that occurs in such cases is not due to mechanical blockage of the renal tubules with precipitated products of haemoglobin. In the case of types (2) and (3) it seems more likely that the presence of large quantities of haemoglobin in the plasma and urine may set up a series of reactions that eventually leads to the situation described, is not unlike that which occurs in blackwater fever and may lead to renal failure. In blackwater fever as much as three-quarters of the blood may be lysed, liberating as much as 600 to 700 grammes of haemoglobin into the plasma, and this may take place during the course of 3 or 4 days, with or without renal failure. No satisfactory data are available to indicate whether oliguria and anuria are commoner in cases where the haemolysis has been large and sudden, than in cases where it is smaller and spread over a longer time. In crush injuries a very small haemoglobinuria may give rise to serious renal failure.

Bearing in mind the severe renal complication that can occur in cases of incompatible transfusion and their similarity to certain cases of blackwater fever, the possibility that some toxic substance may be liberated from the disintegrated erythrocytes should not be overlooked.

From the evidence available in incompatible transfusion it is not clear whether the reactions are due to the haemoglobin, or other unknown factors liberated by the antigen-antibody reaction which occurs.

In cases where the amount of haemoglobin present in the serum is larger and the symptoms delayed, it appears that the circulating haemoglobin may be an important factor in the situation, but toxic products of antigen-antibody reaction should not be ruled out. The fact that various workers have had no serious results from injecting haemoglobin solutions into man may be explained partly by the few cases so far recorded, and the relatively small amounts of haemoglobin given.

In Fairley's experiments (1940) amounts of haemoglobin varying from 180 to 350 mg. per kg. of body weight were injected producing levels in the plasma of between 208 to 230 mmg. per cent. without ill effects. Haemoglobin was present in the urine in his cases when the plasma levels were as low as 37 to 69 mmg. per cent., which is in conflict with the findings of Gilligan and Blumgart (1941), who state that haemoglobinuria occurs only when the

plasma levels reach 100 to 140 mmg. per cent. in the case of march haemoglobinuria. In FAIRLEY's cases there was haemobilirubinaemia; methaemalbumin never appeared in the plasma but Schumm's test was positive, which FAIRLEY regards as being indicative of small amounts of methaemalbumin.

BAYLISS (1920) used a haemoglobin solution produced by alternate freezing and thawing of blood, and injected both stroma and haemoglobin, with no serious consequences. The amount injected into his cat is not stated but would appear to have been approximately 1·0 gramme per kg. of body weight. There was haemoglobinuria.

O'SHAUGHNESSY *et al.* (1939) injected amounts of haemoglobin varying from 10 to 50 grammes in from 200 c.c. to 1,000 c.c. of Ringer's solution: haemoglobinuria occurred but no mention is made of pigment metabolism and no toxic effects followed. OTTENBERG and FOX (1938) injected amounts of stroma-free haemoglobin varying from 4 to 8 grammes into man, which amounts they state are just below and just above that required to produce haemoglobinuria. According to them the rate of injection is of no importance in determining whether or not haemoglobinuria occurs; there is a wide variation in the amount of haemoglobin that different individuals can tolerate before haemoglobinuria manifests itself. They assert that there is a renal threshold for haemoglobin in terms of body weight, which is lower for women than men.

In the dogs of LICHTY *et al.* initial injection of an average of 155 mg. of haemoglobin per kg. of body weight produced no haemoglobinuria; after repeated injections however these authors found that haemoglobinuria supervened from injections of haemoglobin averaging 84 mg. per kg. They also found that haemoglobin is not filtered through the glomerulus at plasma levels lower than an average of 84 mg. per cent. and that iron containing pigment is not deposited in the renal tubule cells when sub-threshold doses are repeatedly injected. At doses above this limit haemoglobin is filtered through the glomerulus, passes down the tubules and is reabsorbed by the cells of the convoluted tubules. This reabsorption continues until the capacity of the tubular cells is exhausted, when haemoglobin appears in the urine.

It seems that the extent of the haemoglobinuria will depend upon the rate at which the haemoglobin containing filtrate is presented to the tubules as well as its concentration and the capacity of the tubules to absorb it. These findings of LICHTY *et al.* in dogs, were confirmed by OTTENBERG and FOX who point out that at high plasma levels the fall in plasma haemoglobin was much more rapid than at low plasma levels; GILLIGAN *et al.* (1941) found that haemoglobin is removed rapidly from the plasma by filtration and as all of it does not appear in the urine, the authors conclude that part is reabsorbed by the tubules, which is in agreement with LICHTY *et al.*

In substance it seems that above certain levels haemoglobin passes through the glomerular membrane, is reabsorbed by the tubular cells until their capacity for further absorption is exhausted, when it appears in the urine (YUILE *et al.*,

1941). These findings coupled with haemobilirubin formation no doubt account for the small proportion of haemoglobin that is found in the urine, when compared with the total number of red cells lysed in cases of blackwater fever.

It should be mentioned that haemoglobin metabolism in dogs, rabbits, horses and sheep appears to differ from that in man, a fact which must be borne in mind when correlating animal experiments with observations on humans. Incubation of a primate plasma-haemoglobin system at 40° C. for 24 hours produces a pigment methaemalbumin with the centre of its α band at 623 m.μ (FAIRLEY and BROMFIELD, 1937; FOY and KONDI, 1938). In plasma-haemoglobin systems of other animals the pigment formed has a haematin-like spectrum (FAIRLEY, 1941) and is not methaemalbumin. Methaemalbumin only forms *in vitro* at pH values of 8·0 or more. Since such values are never attained *in vivo*, there must be different mechanisms of production *in vivo* and *in vitro*.

A further point of difference in pigment metabolism in man, dogs and rabbits is haemobilirubin formation. Normal unhaemolysed rabbits' and dogs' serum gives no reaction with Ehrlich's diazo reagent (FOY and KONDI, 1935; YAMANAKA, 1926; HIDA, 1922; MAEDA, 1932). Dogs with acute haemolytic anaemia from infection with babesia, with blood counts dropping by about 1 million per 24 hours, show no increase in bilirubin until immediately before death when there is a sudden rise; the same occurs in rabbits injected with electro-colloidal copper (FOY and KONDI, *in the press*). In man such haemolysis would cause a considerable rise in the indirect van den Bergh. In view of these facts it appears to us that the interpretation of haemoglobin injection experiments in dogs and rabbits is not fully comparable with that of man and other primates.

MOON (1938, '39, '41, '42), considering the renal changes that take place in such condition as shock, and traumatic injuries, believes that H-Substance liberated from necrosed tissues is sufficient to produce shock, and renal anoxaemia leading to oliguria, azotemia and hypochloraemia followed by the characteristic renal changes described above, which are independent of haemoglobin. Bearing on this aspect of the question HASHIMOTO (1925) has shown that injection of histamine in animals produces diffuse tubular degeneration, a rise in non-protein nitrogen, and marked diminution in urine flow. It seems to us that these observations have an important bearing on the renal failure that sometimes accompanies crush injuries (BLALOCK and BRADBURN, 1930). Other workers have, however, attributed the renal lesion in this condition to local loss of fluid into the injured site, which they claim is sufficient to bring about diminished blood volume and shock with its attendant sequelae.

It is a curious fact that some authors have used haemoglobin solutions that contained stroma as well as the pigment, and had no serious results so far as can be ascertained (*vide* BAYLISS). Others have separated the stroma from the haemoglobin, and injected them separately (MASON and MANN; REID).

Others again have carried out complicated procedures for preparing their haemoglobin solutions, asserting that if this is not done death will occur (BAKER and DODDS). These discrepancies may be due to variations in the amount of haemoglobin given, which a number of authors fail to state precisely (BAYLISS; BAKER and DODDS).

B. HAEMOGLOBINAEMIA IN CONNECTION WITH PERMEABILITY CHANGES.

Whether the presence of haemoglobin in the plasma has any effect on the permeability of the glomerulus or capillaries in general is impossible to say from the evidence so far available.

The passage of substances to and from the capillaries is controlled by a combination of colloid osmotic and hydrostatic pressures, and variations in these bring about the movements of water and electrolytes to and from the plasma (LANDIS, 1934) which are qualitative as well as quantitative. Other factors which change the rate of composition of these fluid interchanges are: (i) anoxaemia, (ii) temperature, (iii) injury either direct or through drugs and hormones, (iv) varying states of hydration and dehydration, (v) nervous impulses, (vi) venous stasis, (vii) muscular activity. Space prevents a consideration of all the factors that affect capillary permeability; any of the above may be operating in conditions of intravascular haemolysis.

The proteins that are abnormally present in the urine it is generally agreed arise from the plasma by passage through the glomerulus. Under normal conditions no proteins pass through the glomerular filter—or only in such small quantities as not to constitute a pathological condition (2·0 to 10·0 mg. per cent.). Under certain conditions, however, protein appears in the urine (nephritis, burns, intravascular haemolysis, shock, crush injury, etc., etc.) and the question arises as to how it passes the glomerular filter, whether by an increase in the permeability of the glomerular membrane thus allowing larger molecules than normal to pass, or through some pathological change in the membrane brought about by unknown factors. The general consensus of opinion seems to favour temporary changes in permeability (FISHBERG, 1939) either produced through toxic action, or as a result of a need to eliminate certain undesirable protein products of metabolism. It has been suggested by WELKER *et al.* (1928) and ANDREWS *et al.* (1929) that the proteins of the plasma may combine with toxic substances in order to render them innocuous, and they are then eliminated by alteration in the permeability of the glomerular capillaries and basement membrane. A somewhat similar view was put forward by CANSTATT (1841). KROGH (1929) has suggested that stretching of the capillary walls may be a factor in permeability change.

[*] It may be mentioned that Bence-Jones protein whenever present is eliminated through the glomerulus although its molecular weight (35,000) is of a size that is generally regarded as large for normal passage. The elimination

of foreign protein is said to "damage the kidney" (*e.g.*, foreign haemoglobin) resulting in "increased permeability" which allows native plasma protein to escape as well. We have little knowledge of the manner by which injury can bring about permeability changes. It is interesting to note that in protein-uric states albumin is the most abundant protein present, with a molecular weight of 68,000 to 70,000; very much rarer is globulin with a molecular weight of 187,000 (SVEDBERG); and fibrinogen, with very much higher molecular weight, is never present in the urine despite the fact that the fibrinogen content of the blood may be relatively high in proteinuria cases. From this and other evidence KERRIDGE and BAYLISS (1932) state that substances are excreted by the kidney according to their molecular weight and radii: the barrier appears to correspond to molecular weights around 68,000 and radii of about 2·5 mμ.

STARR (1926), in a careful series of experiments, has produced some evidence to show that the albuminuria which follows renal vasoconstriction is due to increased glomerular permeability.

DANIELLI (1940) states that the passage of protein complexes through capillaries is due to the existence of pores, and that changes in permeability are due to "clogging" of pores by platelets, which may be purely mechanical. He also points out that one of the actions of normal serum in controlling oedema is that the protein is adsorbed on the walls of the capillary pores. KEYS (1937 and 1938) has come to similar conclusions regarding artificial membranes perfused with serum and gum acacia, and shows that the adsorbed protein can be displaced by substances of higher surface activity (higher polypeptides). The high bile acid content of some plasmas may act in the same way. DANIELLI states that dilated pores are not readily permeable to molecules whose weight is over 100,000 with diameters corresponding to 6 mμ.

The proteinuria that occurs in the haemoglobinurias of the intravascular haemolyses has not been very carefully investigated so far. GILLIGAN *et al.*, carrying out haemoglobin injection experiments in man similar to those of FAIRLEY, state that "albuminuria" occurred in all cases when enough haemoglobin was injected to bring about a haemoglobinuria, and that the proteinuria was higher than could be accounted for by the globin portion of haemoglobin in the urine. Patients with pre-existing albuminuria had a lower renal threshold for haemoglobin. Such albuminuria and haemoglobinuria may be accounted for by assuming increased permeability of the glomerular membrane; but toxicity could not be ruled out as a factor in bringing about such permeability variations.

KERKHOFF (1937), commenting on capillary permeability changes in oedematous states, says that any such changes that take place are unlikely to be local, but general. If haemoglobin free in the plasma has any effect on capillary permeability it is unlikely that it would affect only the glomerular capillaries, although LANDIS (1934) and LANDIS *et al.* (1935) and KROGH have shown that there are variations in capillary permeability to dyes in different capillary

networks. GERSH (1936), using foreign carboxy haemoglobin, states that excretion of haemoglobin temporarily injures the glomerular membranes and thus allows the passage of larger molecules than normally. HESSE and FILATOV, using homologous haemoglobin, noted that in their dogs albuminuria preceded haemoglobinuria, thus indicating that glomerular changes can be caused by haemoglobin.

MONKE and YUILE state that 3 per cent. of the pores in the glomerulus are large enough to allow the passage of undissociated haemoglobin molecules with a weight of 68,000. According to those authors, the pores carry electric charges as a result of which the effective diameter of the pore is less than its structural when filtering through large charged molecules like haemoglobin. Thus the electric charge on such large molecules becomes a factor in their filtration, and is itself dependent upon the isoelectric point of the transudate and the pH of the solvent. That haemoglobin injures the glomerular membrane is not regarded as a factor by MONKE and YUILE, on account of the constancy of the haemoglobin threshold, and the regularity of the clearance curves. It should be mentioned here that all workers have not found the same or constant thresholds for haemoglobin (cf. FAIRLEY, GILLIGAN *et al.*, OTTENBERG and FOX). KEYS suggests that it is not necessary to postulate the existence of permanent gaps in the membrane since transitory ones would serve.

If 3 per cent. of the pores in the glomerular membrane are allowing undissociated haemoglobin molecules to pass, it is surprising that more protein is not found in urine normally, but this may be on account of a different electric charge or isoelectric point. MONKE and YUILE do not consider that haemoglobin dissociation (see below) is a factor in its passage through the glomerulus, since they state that drastic conditions are necessary for such dissociation. TISELIUS and GROSS (1934), however, point out that haemoglobin dissociates into half-sized molecules in dilute solutions; and STEINHARDT (1938) states that isoelectric horse haemoglobin is completely dissociated into molecules of half the normal molecular weight when high concentrations of urea or other amides are present in the solution. We wonder whether the azotemia present in all the conditions we are dealing with has any bearing on this aspect of the question.

SVEDBERG (1930) considers protein molecules fall into two groups according to their molecular weight—one group inlcuding molecules whose weight ranges from 34,500 to 208,000, and the second group with molecular weights of the order of millions, like haemocyanin (5,000,000). He points out that the first group have weights that are multiples of 34,500 ($\times 1$, $\times$ 2, $\times$ 3, $\times$ 6), and that they are stable over a wide range of pH; haemoglobin, for example, being stable over a range of pH 6 to 8. Proteins having weights that are multiples of 34,500 can generally break down into their submultiples, haemoglobin can split into $4 \times 17{,}000$ (or $1{\cdot}97 \times 34{,}500$, which is nearly the theoretical multiple

of 34,500 × 2). These conclusions have, however, been criticized by SCHMIDT (1932).

YUILE and CLARK (1941) and YUILE *et al.* (1941), on the basis of their studies on myohaemoglobinuria, state that the limit of glomerular permeability is approximately that of the inulin molecule (M.W. 15,000). Bence-Jones protein, however (M.W. 35,000) normally passes through the glomerulus, presumably without damaging it.

BOTT and RICHARDS (1941) have shown by glomerular puncture in the Amphibia that molecules with a weight of 35,200 will pass through the glomerulus but that differences exist between individual proteins; they state that most of the " mesh " will pass particles with a diameter of 20 Å (2 mμ.) and only half of the " mesh " will allow the passage of particles with a diameter of 50 Å (5 mμ.). They agree that the important factor in determining passage is molecular size, but do not rule out other factors.

In view of these facts there appear to be two possible explanations to account for the passage of haemoglobin through the glomerulus and its appearance in the urine : (*a*) the glomerulus normally possesses a small proportion of pores sufficiently large to permit the passage of undissociated haemoglobin molecules (M.W. 68,000) ; (*b*) that a certain number of haemoglobin molecules may dissociate $Hb4 \rightleftarrows Hb2 \leftrightarrows Hb1$. Such dissociation of haemoglobin taken in conjunction with the view that the glomeruli are normally permeable to molecules of a molecular weight of 15,000 (inulin) and 35,000 (Bence-Jones protein) would suffice to account for haemoglobinuria. If either of these explanations is correct then there is no need to postulate glomerular injury due to toxicity or changes in permeability. The possibility that all three mechanisms may operate together must not be forgotten.

Summary.

It appears that haemoglobin in sufficient quantity in the plasma may have a toxic effect on metabolism in general and on the kidney in particular. The absence of effects that some workers have reported from their haemoglobin injections may have been due to the relatively small amounts of haemoglobin used, and the small number of cases so far reported. The toxic effects described by other authors may have been due to other factors than haemoglobin, and to such complications as the administration of ammonium chloride, bicarbonates, etc. In most of the cases there appears to have been dehydration, haemoconcentration and electrolyte-acid-base disturbances which are known to be important in the formation of urine (see below).

The severe reaction that follows the injection of small amounts of incompatible Landsteiner groups would seem to fall into another category. The series of events taking place when larger quantities of blood, containing the Rh. and other subgroups, are injected seems to resemble the situation in black-

water fever, with the difference that in the latter, patients' as well as donors' cells are lysed ; and the symptoms may be due to haemoglobin, or other unknown factors liberated by the antigen-antibody reactions.

The exact nature of the changes in the glomerular membrane that permit the passage of haemoglobin molecules is at present uncertain. There may be a small number of pores in the glomerulus that allow the passage of undissociated haemoglobin molecules with a weight of 68,000. If SVEDBERG's view that proteins can break down into sub-multiples of their molecular weight is correct, this would permit Hb2, with a molecular weight of 34,500, to pass in the same way as Bence-Jones protein does normally. If either of these views is correct it is unnecessary to postulate either glomerular injury of which there is no anatomical evidence, or permeability changes, about the nature of which little is known, and often serves as a cloak for ignorance. The problem of electric charges on molecules and isoelectric points may also be factors determining what molecules shall pass the glomerulus.

The work of DANIELLI shows that platelets by mechanical means may bring about permeability changes by sticking to the pores crosswise and hindering the passage of substances: this may also be a factor. Nervous impulses cannot be ruled out as a means of controlling permeability.

It is probable that a combination of forces are at work, and no single explanation can account for all the facts.

C. ELECTROLYTE, ACID-BASE, AND WATER BALANCES.

" One of the most jealously guarded constants of the organism is the total electrolyte concentration of the blood plasma " (PETERS and VAN SLYKE, 1931) and the chief means of maintaining this electrolyte balance is the kidney and, in the case of chlorides, as has been shown by IVERSEN and HANSBORG (1922), diffusion into the tissue spaces. In addition the diffusion of water between the extracellular fluids and the cells also assists in the maintenance of electrolyte and water balance ; a dilution of the blood plasma, for example, causes the red blood cells to swell by taking up water, thus diminishing the dilution of the remaining electrolytes (PONDER, 1934). The plasma pH will remain within physiological limits provided the balance of anions (Cl, HPO_4, HCO_3, protein, etc.), and cations (K, Na, Ca, Mg, etc.) is not seriously disturbed. And, moreover, a depletion of chlorine ions as may occur in vomiting can to a limited extent be balanced by increases of other anions and by loss of base.

Very little is known concerning the effects of gross changes in the blood cations, probably because it is so difficult to bring about such changes experimentally, except in the case of calcium. Whenever such changes do, however, occur the results are generally profound.

Gross dilution of the plasma with water given at a rate that is faster than

the kidney can eliminate it, produces toxic results, as has been shown by ROWNTREE (1923 and 1926) and MISAWA (1927).

Chlorides are an especially important entity in the maintenance of the neutrality of the body fluids, and uncompensated diminution gives rise to toxic symptoms that some authors regard as due to hypochloraemia *per se.* (BLUM *et al.*, 1929a.)

A considerable amount of evidence has recently been produced to show that variations in electrolyte balance in the blood can exert a profound effect on renal function (PETERS and VAN SLYKE). It has also been shown that hypochloraemia, acidosis and alkalosis frequently lead to extra-renal azotemia, and are accompanied by reduction in glomerular filtration as estimated by creatinine and inulin clearances, which is regarded by MCCANCE and WIDDOWSON as the chief cause of the uraemia. (BROWN *et al.*, 1923; BLUM *et al.*, 1929a and b; VAN CAULAERT and PÉTREQUIN, 1931; PORGES, 1932; GOMORI and PODHRADSZKY, 1937; GOMORI and FRENREISZ, 1937; MCCANCE, 1936 and 1939; KERPEL-FRONIUS, 1936a and b; HARDT and RIVERS, 1923; BERGER and BINGER, 1935; COPE, 1936; STEELE, 1936; MCCANCE and WIDDOWSON, 1937; NICOL, 1940; WILKINSON and MCCANCE, 1940; HADEN and ORR, 1923a, b, c and 1928.)

BAKER and DODDS assessed the kidney function in their experimental rabbits by rising blood ureas, but gave no data concerning the sodium chloride levels and output of the blood and urine. Further, it is uncertain at the moment whether glomerular filtration processes are the same in rabbits as in man.

LANDIS and his co-workers have shown that in individuals kept on a known constant nitrogen diet with adequate fluids, the 24-hour clearance of urea varied directly with the sodium chloride level. From their experiments the authors conclude that neither dehydration nor oliguria *per se* could explain the changes in renal function since their cases took on an average 4,000 to 5,000 c.c. of fluid per 24 hours and excreted about 3,000 c.c. They say that chloride levels in blood have an important bearing on renal function which is independent of fluid intake. GAMBLE (1929 and 1936) also found that sodium chloride restriction reduced urea clearance by about 60 per cent. CHAUSSIN (1920) found that when fluids are restricted, and sodium chloride administered the excretion of urea is diminished. ADOLPH (1923) points out that normally the total osmotic concentration of the urine does not exceed a fixed value, and that if the concentration of one substance increases unduly, the excretion of the other substances will be correspondingly depressed to maintain the total osmotic pressure within limits. LANDIS *et al.* confirmed these findings, but in addition state that loss of chlorides produces dehydration, anhydraemia, with reduced plasma volume, and increased plasma proteins.

BANG (1915) has also shown that if an animal is deprived of both food and water the non-protein nitrogen rises more than it does when deprived of food only. From a number of experiments it appears that water deprivation

alone may be responsible for a rise of non-protein nitrogen. (Cf. MORGULIS and EDWARDS, 1924; MACKAY and MACKAY, 1924.)

GLASS (1932), in a careful series of experiments lasting over 20 days, produced hypochloraemia without dehydration. In his experiments there was an early rise in urea, which the author attributed to increased protein catabolism. This was followed by a terminal uraemia associated with upsets in renal function, which could be overcome by the administration of sodium chloride. GLASS considers that since the sodium chloride had to be reduced by 30 per cent. before symptoms developed the dehydration is more important than hypochloraemia.

GROAK (1936) and CLAUSEN (1937), injecting urea in large amounts, produced azotemia without any change taking place in blood sodium chloride, and are of the opinion that chloride levels do not directly affect nitrogen excretion. BAYLISS and BROWN (1940) state that the excretion of water is independent of chlorides but sodium may have been a factor in their experiments.

KERPEL-FRONIUS (1936a) believes that dehydration is the important factor since urea can only be excreted when there is water in which it can be eliminated.

Later, KERPEL-FRONIUS and BUTLER (1935) showed that in rabbits with azotemia due to salt restriction the important ion was sodium and not chloride; that hypochloraemic animals with normal blood sodium were neither dehydrated nor azotemic, but that those with low blood sodium but adequate water intake were both dehydrated and azotemic. GOMORI and PODHRADSZKY, and GAMBLE confirm this view, and state that in their opinion hypochloraemia plays no direct part in onset of azotemia, but that sodium deprivation, by leading to dehydration, is responsible. Thus in the opinion of these workers sodium depletion leads to dehydration that is independent of fluid intake, and this, in turn, leads to azotemia. Following dehydration there is haemoconcentration, and decreased blood flow through the kidneys (MEDES and HERRICK, 1933) and reduced glomerular filtration. It is not impossible that dehydration may give rise to an increased blood protein and osmotic pressure which would additionally hinder filtration (JEGHERS and SELESNICK, 1937).

These workers also state that BLUM'S view that the azotemia present in extra-renal conditions is compensatory, and due to hypochloraemia, is without foundation. They state, however, that there is a consistent relation between dehydration and azotemia, which is related to the dependence of urea clearance on the volume of the urine as described by MÖLLER *et al.* (1928).

FOLLIS and his collaborators (1942) have shown that rats on a deficient potassium diet develop gross changes in the epithelium of the renal tubules similar to those described in crush injuries.

The fact that many workers have concentrated their attention on individual ions is apt to cause confusion, since electrolytes are not always lost in the same proportion in the various body fluids. Much of the confusion that exists in

this field is undoubtedly due to this limitation of observations instead of studying the electrolyte and water balance as an interrelated whole. A patient who vomits, for example, loses much chloride as hydrochloric acid, but little sodium, this chloride may be replaced by bicarbonate. Loss of pancreatic fluid on the other hand causes marked hyponatraemia, with little change in blood chloride (JEGHERS and BAKST, 1938). Sodium and chloride cannot therefore be separated from one another and acid-base balance in this discussion.

In blackwater fever WAKEMAN *et al.* consider that the initial functional disturbances (shock, diarrhoea and vomiting) provide unfavourable conditions for the secretion of urine. The vomiting and anoxemia, with consequent salt depletion, is likely to lead to electrolyte imbalance dehydration, changes in blood volume and viscosity, and thus prejudice glomerular filtration, not to mention the little understood effects of the presence of large amounts of haemoglobin free in the plasma. Oliguria and anuria are much commoner in those cases of blackwater fever where vomiting and diarrhoea are present leading to salt and water depletion, decreased glomerular filtration, oliguria, anuria and azotemia.

MCCANCE and WIDDOWSON, discussing why hypochloraemia should lead to an increase in the reabsorption of urea, state that in their opinion the main cause of the "uraemic" condition is reduced glomerular filtration which is not produced by fall in blood pressure. They suggest that in man increased osmotic pressure of the plasma may be important, or reduction in the number of functioning glomeruli owing to diminution of blood volume (cf. EGGLETON) or diminished renal circulation on account of blood viscosity and, lastly, unknown factors. In connection with viscosity it should be noted that decrease in number of circulating erythrocytes by haemolysis would tend to reduce viscosity, and this may balance any increase due to dehydration (BURNS, 1929).

Whatever may be behind the azotemia that develops after the injection of haemoglobin solutions into animals, it is not necessarily due to blockage of the renal tubules with haemoglobin products. And whatever the final conclusion regarding the cause of extra-renal azotemia, it is evident that electrolyte and water balance is a factor of importance, and has a close association with renal function, and disturbances of this balance may lead to the production of oliguria, anuria, as well as other renal changes.

As has been pointed out above, the anuria that sometimes occurs in the intravascular haemolyses has been attributed by some authors to blockage of the renal tubules as a result of precipitation of haemoglobin and its derivatives from an acid tubular filtrate (below pH 5·5), sodium chloride concentration playing a subsidiary role. It has been suggested that this could be prevented by the simple process of alkalization, thus ensuring the secretion of an alkaline urine. The problem of acid-base balance and its relation to the reaction of the urine is by no means a simple matter, and recent work has tended to show

that not only is alkalaemia prone to develop while the urine is still strongly acid in reaction, but that the indiscriminate use of alkalies in renal conditions is not without danger. It might also be added that so far as statistics are available there is no evidence to show that oliguria or anuria is less common in the intravascular haemolyses now than before the introduction of this therapy. There are very few cases of blackwater that do not receive alkaline treatment in the hands of physicians, yet so far as we are aware the death rate in anuric cases of this disease has not fallen.

Alkalosis can itself not only depress renal function and reduce glomerular filtration as shown by inulin clearance tests, but also depress the excretion of solids (McCance; Ziegler *et al.*, 1937). It is not uncommon to see patients with peptic ulcer treated with alkaline powders, who increase the dose with every recurrence of pain with the eventual result of coma, blood examination showing high plasma bicarbonate, and blood urea: everything returning to normal when the alkali treatment is stopped (Kirsner and Walker, 1942).

Wakeman *et al.* state that there was no evidence of acidosis in their cases of blackwater fever, and therefore no need for alkaline therapy. Further acid-base disturbances in nephritis have always been regarded as a consequence of renal failure and not a cause. In the conditions we are describing here, acid-base balance fluctuations are probably secondary to anorexia, vomiting, diarrhoea, heroic doses of citrates, bicarbonates, and in animals ammonium chloride. In the case of anuria and oliguria van Slyke suggests intravenous or subcutaneous saline as the most effective therapeutic measure, and by this means it may be possible to prevent anuria.

Obviously one of the important factors in the production of urine is fluid intake, and if this is restricted the output of urine will be reduced. Considerable quantities of fluid are eliminated from the body by way of the skin and lungs and fluid intake must be sufficient to balance the losses by all these routes. The normal fluid loss amounts to 2,800 to 3,000 c.c. per 24 hours, more or less equally divided between the kidneys, the skin and lungs. If there is fever, more is lost *via* the skin and less *via* the kidneys with a consequent secretion of concentrated urine. Even when the kidneys are concentrating urine to a specific gravity of 1,032, 500 c.c. of water are required in which to excrete the 35 grammes of waste produced (Lashmet).

This aspect is of importance in regard to the formation of tubular plugs because where the amount of water available is limited and the amount of solids normal, or increased through fever, etc., the concentration of the urine will be increased and the separation of precipitates more likely. The point at issue is whether the plugs primarily produce the renal failure or if their production is a parallel phenomenon due to antecedent disturbances. The haemoconcentration that results from dehydration, whether produced by vomiting and/or diarrhoea, may lead to a decrease in glomerular filtration and a reduction in urine flow. Haemoconcentration can result from other causes than simple

loss of fluid ; it can be physiological in the sense that acidosis and alkalosis, as well as plasma protein changes, may produce conditions simulating dehydration because the excretion of solutes demands water (MARRIOTT, 1923 ; KEITH, 1924 ; HARTMANN, 1928 ; PETERS, 1932, 1934, 1935 ; MEYLER, 1936).

One of BAKER and DODDS' rabbits was given green food and produced alkaline urine, the other oats and bread with "free access to water" and produced acid urine. In neither case did anuria develop after the injection of haemoglobin, but in the second rabbit azotemia did.

YORKE and NAUSS had previously found that rabbits on green food could tolerate large quantities of intravenous haemoglobin without developing anuria, whereas another group on oats and bread without access to water passed an acid urine and subsequently became anuric. They stated that the anuria could be prevented by saline injections. ROSS also agrees with YORKE, stating that adequate water intake is of greater importance than any other form of therapy. LINDAU (1928) also considers that the debris in the tubules is secondary to a diminishing urine flow. GERSH, injecting pig haemoglobin into rabbits, some of which were dehydrated, found that the kidneys of the dehydrated animals contained vastly greater amounts of granular debris and haemoglobin than did the non-dehydrated animals ; in this respect they resembled his low blood pressure animals. As stated above, RIGDON and CARDWELL, NICOL, and WAKEMAN *et al.* agree that water deprivation is the most important factor in causing tubular degeneration, and that if steps are taken to maintain an adequate water intake large quantities of haemoglobin can be injected without any ill effects as was earlier shown by YORKE and NAUSS.

In these studies there seems to have been general agreement that glomerular filtration is reduced as a result of one or more factors that may be acting on the electrolyte-acid-base-water-balance, and that dehydration is one of the most important in the complex and indicates the preponderating importance of upsets in water balance in the production of oliguria and anuria, and failure to recognise this has often clouded the issue.

Summary.

Electrolyte disturbances such as are likely to occur in intravascular haemolyses have an important effect on renal function and, acting in conjunction with acid-base-water-balance disturbances, affect blood volume, viscosity and flow. These factors, acting singly or in combination, appear to have an effect on glomerular filtration rates, resulting in a diminution in the flow of urine, inadequate flushing, oliguria and azotemia. Whether hypochloraemia *per se* can bring about these results is not yet fully established, but that electrolytes in general, and perhaps chlorides in particular, have an extremely important bearing on renal function, seems to have been established. The relative importance of glomerular filtration and tubular reabsorption is discussed later.

D. Colloid Osmotic Pressure in Relation to Glomerular Filtration and Tubular Reabsorption.

As pointed out below, physical forces are an important factor in regulating fluid movement through capillary walls, and hydrostatic pressure in the capillaries is the chief means of controlling transudation when all other factors remain normal.

The total osmotic pressure of blood is about seven atmospheres (about 5,000 mm. Hg.) due almost entirely to the electrolytes present in the blood. Since these are easily diffusible they exert no effective osmotic pressure. The colloids, on the other hand, exert an osmotic pressure that is equal to about 30 mm. Hg., a force that is more or less balanced by the hydrostatic pressure originating from the heart pump. The hydrostatic pressure in the glomerular capillaries is in excess of that of the other capillaries, and is estimated at approximately 45 to 100 mm. Hg. (Hayman, 1940). In cases where the hydrostatic pressure exceeds that of the colloid osmotic pressure fluid will pass out of the capillaries; similarly, falls in hydrostatic pressure and/or rises in colloid osmotic pressure will result in fluid being retained in the capillaries.

It has been shown that it is not the fall in the proteins as such that causes fluid loss from the vessels but the osmotic pressure that they exert, since if the plasma is replaced by some other osmotically active substance like gum acacia there is no loss of fluid from the vascular system (Fahr and Kerkhof, 1933). Goudsmit and his collaborators (1940, 1941), and Lepore (1937) have, however, denied that the gum acacia exerts its effect by alterations in osmotic pressure but, through its effect on sodium chloride, metabolism and blood volume increase ; their conclusions have, however, been criticised (*Lancet*, 1942 ; cf. Danielli and Keys).

The question arises as to whether the physical changes that take place in the blood stream in the acute intravascular haemolyses are in any way connected with the reduction in glomerular filtration, oliguria, anuria and azotemia. Very little work has so far been done on this aspect of the question. A 50 per cent. haemolysis is by no means uncommon in blackwater fever, and represents an addition of some 8 per cent. of protein (Hb) to the plasma, which should result in an appreciable rise in osmotic pressure. Foy and Kondi (1938) found that in blackwater fever the total serum proteins were normal, in spite of the large additional amount of haemoglobin thrown into the plasma by the haemolysed blood cells. In our view this points to a reduction in the normal serum albumin and globulin in blackwater fever, which is about equivalent to the protein liberated from the lysed red cells, a view that is borne out by the large amount of protein other than haemoglobin that is found in the urine. Increased colloid osmotic pressure then does not appear to contribute to the diminished glomerular filtration and oliguria of blackwater fever.

Variations in hydrostatic pressure may, however, be a factor in this disease.

No very systematic studies have been made on blood pressure in blackwater fever (STEPHENS, 1937). In some cases it sinks to levels that are incompatible with the proper secretion of urine (70 to 75 mm. Hg.). It must, however, be remembered in this connection that small changes in systematic pressure can be compensated for by the renal capillaries, so that although the pressure in the renal capillaries may vary in the same general direction as systematic pressure, the latter is not a true measure of the renal situation (STARLING, 1896; FAHR and ESSHLER, 1941). A consideration of recent work makes it appear likely that variations in glomerular filtration are more intimately bound up with renal blood flow than with blood pressure (RICHARDS and PLANT).

A fall in blood pressure associated with unchanged osmotic pressure of the plasma may find the kidneys unable to make the necessary compensation. FAHR and KERKHOF have shown that rises of as little as 7 to 8 mm. of Hg. in the hydrostatic pressure of the capillaries result in oedema, and it is not impossible that similar decreases unaccompanied by compensatory changes in osmotic pressure of the plasma may cause variations in glomerular filtration and reduce urine output.

Summary.

Before any conclusions can be reached concerning the effect of variations in colloid osmotic hydrostatic pressures much more information is necessary. In cases of shock and incompatible blood transfusion, falls in blood pressure would appear in many instances at least to produce sufficient changes in glomerular filtration, and this in conjunction with factors already mentioned may play a part in the onset of renal failure.

E. CHANGES IN GLOMERULAR FILTRATION AND TUBULAR REABSORPTION.

All the factors so far discussed are those which operate to reduce glomerular filtration and thus upset urinary secretion by affecting factors in the plasma that will reduce the volume of the filtrate. A second factor likely to cause variation in urinary output is tubular reabsorption. BYWATERS and DIBLE (1942) have suggested that in crush injuries excessive unselective reabsorption of the filtrate by damaged tubules may be a factor more likely to contribute to anuria than is mechanical blockage. As mentioned above, HASHIMOTO has noted extreme tubular degeneration after histamine injection and this would seem to fall in with the state of affairs in crush injuries and link up with the ideas of MOON and others regarding H-substance as the toxic agent in such conditions. NICOL, and RIGDON and CARDWELL, as noted above, described tubular changes in all their experiments. AYER and GAULD (1942) pointed out that in their post-transfusion uraemias, where the kidneys have been closed down for 3 days or more, a brick-red and brownish material occurred in the cytoplasm of the epithelial cells of the distal convoluted tubules, and collecting

ducts. These workers also mention the clogging together of masses of epithelial cells which later degenerated. The chemical nature of the material forming the casts in post-transfusion renal insufficiency has not been determined with any accuracy. There is strong presumptive evidence that it is a haemoglobin derivative. It is impossible to state how much of the material found in the tubules is derived from haemoglobin and how much from degenerated epithelial cells. The distribution of pigment described by AYER and GAULD is different from that described by YORKE and NAUSS, and BORDLEY, and they state that necrosis of the epithelial cells only occurred in those parts where there was intra-cellular red material and that the brown material was quite innocuous.

The colloidal droplets and degenerative changes seen in the tubules of nephrotic cases are, according to JOHNSON and SMETANA (1941), due to reabsorption and storage of proteins that have passed through the glomerulus. SMITH also suggests that the engulfing of material (athrocytosis) by the epithelial cells may be a factor in their degeneration.

LICHTY *et al.* have shown that haemoglobin is taken up by the tubular cells until these become incapable of taking up more. The inclusion of such haemoglobiniferous products in the plugs affords a ready explanation of the descriptions of these plugs as consisting of precipitated haemoglobin. FISHBERG considers that at the moment there is insufficient evidence for concluding that some toxic substance is responsible for the tubular changes.

DE GOWIN and BALDRIDGE described two cases of fatal renal insufficiency following incompatible blood transfusion: the postmortem findings revealed reddish-brown granular pigment in the lumina of the tubules together with necrosis of the tubular epithelium. WITTS (1929) found haemoglobin infarction in the tubules in his case of anuria following incompatible transfusion. Similar findings have been described in other cases of incompatible transfusion (GOLDRING and GRAEF, 1936; VAN DEESTEN and COSGROVE, 1933; JOHNSON and CONWAY, 1933; BORDLEY, 1931; DE GOWIN *et al.*, 1937 and 1938; DE NAVASQUEZ, 1940; RAVID and CHESNER, 1940; etc.). In crush injuries a like state of affairs has been reported, together with necrosis of the epithelium in Henle's loop (BEALL *et al.*, 1941; BYWATERS and BEALL, 1941; DUNN *et al.*, 1941; MORISON, 1941; BYWATERS and DIBLE, 1942). In blackwater fever the findings are the same (YORKE and NAUSS, 1911; STEPHENS, 1937).

WAKEMAN *et al.* in experiments on dogs given injections of haemoglobin, say that occlusion of the renal tubules is the most important single factor in the onset of anuria, and quote the results of their experiments on five dogs which were given haemoglobin either intravenously or intraperitoneally in amounts of 2 to 3 grammes per kg. of body weight. The two that received their haemoglobin intravenously recovered, they did not suffer from anuria, and when killed showed " a few casts in the collecting tubules." Of the three receiving their haemoglobin intraperitoneally two showed extreme blood concentration (high cell-plasma ratio), vomiting, oliguria, lowered serum chlorides

and bicarbonate. At postmortem these animals showed pigment casts and plugging of the whole tubular system.

The authors attribute the oliguria and anuria to the blockage of the renal tubules, but in our view a more reasonable explanation is the diminished flow of urine from the shock caused by the intraperitoneal injection as evidenced by the extreme haemoconcentration and fall in blood pressure. The state of the kidney can be better explained on the basis of decreased glomerular filtration resulting from shock. It is difficult to say exactly what would be the result of such changes in the tubular epithelium on the eventual composition of the urine. FISHBERG argues that when necrosis is marked no reabsorption can be expected and a dilute urine should result. BYWATERS and DIBLE, on the other hand, suggest the possibility of " excessive unselective reabsorption "; this could lead to oliguria and anuria but hardly to the excretion of a concentrated urine.

All these workers seem to agree that tubular changes, sometimes gross, are present in the conditions that they describe. Exactly how they are produced is not clear and how far reduced glomerular filtration may be a factor in their onset or other unknown causes is at present impossible to say. It does appear likely that these tubular changes are often sufficiently severe to interfere with the normal processes of tubular reabsorption, urine formation and concentration. The changes that take place in the volume and specific gravity of the urine in the acute intravascular haemolyses indicate that some radical changes have taken place not only in the filtration mechanism but also in the reabsorption. Since the osmotic pressure of the urine when determined in these cases is always higher than that of the plasma, osmotic work is clearly being done by the tubules and therefore the " damage " so consistently reported by histological examination cannot be sufficient in extent or degree to derange completely the concentrating power of the kidneys. And it is not unreasonable to suppose that the acid-base-electrolyte-water-balance discussed above will affect the processes of reabsorption as they do that of filtration.

Under normal conditions variations in glomerular filtration are said not to be a factor in bringing about variation in the urine volume (WHITE and WINTON). Experiments in dilution diuresis on isolated kidneys and on anaesthetized dogs show that small changes in colloid osmotic pressure cause a disproportionally great diuresis when compared with that produced by an equivalent rise in blood pressure, and EGGLETON *et al.* suggest that in this form of experiment there is some direct action on the tubules which inhibits the reabsorption of water, coupled with an increase in glomerular filtration. BLACK *et al.* (1941), determining the clearances of inulin and perabrodil as measures of glomerular filtration and renal blood flow, consider that in their cases of extra renal azotemia due to haematemesis, the fall in glomerular filtration was due to diminished plasma flow to the filtering surfaces of the glomeruli, and

that this in turn was due to diminished blood volume and pressure, and other circulatory disturbances accompanying shock.

It is probable that the changes found in the tubules at necropsy are not to be attributed to any one factor; the physical and chemical disturbances discussed above which affect glomerular filtration will also no doubt affect the tubules. In addition, the taking up of granular material has been suggested by several authors as one of the causes of the epithelial degeneration, and most authors seem to agree that the tubular cells contain abnormal pigments prior to their degeneration. Reduction of blood flow is more likely to affect the tubules than the glomeruli, since the amount of work done by the former and the oxygen consumed is vastly greater than by the latter.

F. Urinary Pigments in Relation to Blockage.

The presence of abnormal amounts and types of substances in the glomerular filtrate and their precipitation in the renal tubules has been held to give rise to blockage leading to anuria and azotemia. As we have already pointed out, this explanation seems to us to be putting the cart before the horse, in that decreased glomerular filtration and/or abnormal reabsorption processes result in diminished flow of urine, and this, together with an increase in the amount of electrolyte to be excreted, and the tubular changes in such conditions as blackwater fever, may lead to the formation of a concentrated urine.

The pigment present in the urine in the various haemolytic conditions has only been carefully investigated in blackwater fever, and even here our knowledge of the chemistry of the various pigments present is extremely fragmentary. There is fairly definite information that in blackwater fever and paroxoysmal haemoglobinuria, both haemoglobin and methaemoglobin occur in the urine. In crush injuries myohaemoglobin with a molecular weight of 17,500 is reported (Bywaters *et al.*, 1941).

There can be little question that the haemoglobinuria results from haemoglobinaemia, but the situation is not so precise regarding methaemoglobin in the urine. Baker and Dodds assumed that the methaemoglobin was derived from haemoglobin during its passage down the tubules, pH variations being the important factor in the conversion.

Ross (1932) has suggested that there may be two possible explanations for the urinary methaemoglobin: (1) the methaemoglobin in the plasma being filtered through the glomerulus and appearing unchanged in the urine; (2) conversion of the haemoglobin filtered through the glomerulus into methaemoglobin during its passage down the tubules or its stay in the bladder. It is now known that methaemoglobin never appears in the plasma in blackwater fever (Fairley and Bromfield, 1937; Fairley, 1941; Foy and Kondi, 1938), but that the pigment present in the plasma having the centre of its

α band in the red at 623 mμ. is not methaemoglobin but methaemalbumin and that this pigment never appears in the urine. There also appears to be no direct quantitative relationship between methaemalbumin in the plasma and methaemoglobin in the urine (Foy and Kondi). We can therefore, in the present state of our knowledge, rule out the first suggestion of Ross that the methaemoglobin of the urine is derived from a similar pigment in the plasma.

Ross's second suggestion that the methaemoglobin in the urine may arise from changes in the haemoglobin in the filtrate as it passes down the tubules, or while it is in the bladder, seems at the moment to be the only alternative. Although Ross agrees with the main contentions of Baker and Dodds that pH has an important bearing on the types of pigments present, he points out however that methaemoglobin is found in urines that have a pH as high as 7·0 and that time is an important factor in the conversion of oxyhaemoglobin into methaemoglobin. Wright (1940) states that the change from oxyhaemoglobin to methaemoglobin goes on over a pH range of 5·0 to 9·0. In addition, Fairley (1940) has shown that in plasma-haemoglobin systems incubated at 37° to 40° C. alkaline methaemoglobin only forms if the pH rises above 8·0. It must be admitted, however, that the question of the chemistry of haemoglobin during passage through the kidney and in the urine is little understood at present, and that *in vitro* experiments are not necessarily a true picture of what may be going on *in vivo.* The discrepancy between Baker and Dodds' conclusions may be to some extent due to this factor.

Ross considers that time is the important factor in the conversion of haemoglobin into methaemoglobin and states that the lower the pH of the urine the shorter the time required for the change to take place, and mentions the longer the time that elapses between the entry of the urine into the bladder and its examination the greater is the chance of finding methaemoglobin. In a case of paroxysmal haemoglobinuria (Foy and Kondi, *in the press*), we had the patient put her feet in cold water for 45 minutes, she then passed about 12 c.c. of black urine loaded with haemoglobin and methaemoglobin, with a pH of 5·4. Since the bladder was emptied immediately before the chilling and only 45 minutes elapsed between the passage of the two lots of urine, it seems to us that the conversion probably took place during passage down the tubules.

Although methaemalbumin occurs in the blood it has never been detected in the urine, so either it does not pass the glomerulus or is completely absorbed by the tubules, as is haemoglobin, or changed into some other derivative of haemoglobin during its passage. The possibility that it does not pass the glomerulus should not be excluded since it has been found that this substance does not pass from the maternal blood supply through the placenta to the foetus (Foy and Kondi, 1941). Its molecular weight (68,000 to 70,000) is of a size that should permit it to pass through the glomerulus if undissociated

haemoglobin passes; here again its isoelectric point and charge may be different from that of haemoglobin and so prevent it from passing through the glomerular pores. The concentration of methaemalbumin in the plasma is never very high.

An important factor in the question of precipitation is the concentration of haemoglobin present in the filtrate, which will in turn be dependent upon the volume of fluid and the magnitude of the haemolysis. A small haemolysis may produce just as large a precipitate if the volume of the fluid available to take it up is limited in amount. Hence the importance of adequate fluid intake in all forms of haemoglobinuria.

ROSS considers that BAKER and DODDS' view that sodium chloride concentration is an important factor in the precipitation of haemoglobin has been over-emphasized, since he found that in his cases of blackwater fever rarely was the concentration 1 per cent., and that in his suppression cases it was only 0·4 to 0·5 per cent. WAKEMAN *et al.* report the same findings. We would suggest that total electrolyte concentration is as important, or more so, since it would seem to us that if precipitation occurs it is more likely to take place from highly concentrated urines.

The question of the origin of methaemoglobin in the urine is by no means a simple one. What factors are at work in converting the oxyhaemoglobin into methaemoglobin as the urine passes down the tubules is not clear, mere changes in pH would not seem to be the only mechanism involved. As reported by YORKE, ROSS, and BYWATERS *et al.*, the conversion may go even further than methaemoglobin, and result in the formation of haematin which is then taken up by the tubules or deposited in the lumina. What proportion is converted into haematin has not yet been determined. It is not without interest to note that in some cases of blackwater fever only methaemoglobin is present in the urine, in others oxyhaemoglobin and methaemoglobin, and yet in others only oxyhaemoglobin. The reason for these differences is not to be found in simple pH changes. In crush injuries met-myohaemoglobin and oxyhaemoglobin have been reported, but although the urine was acid there is no methaemoglobin present so far as can be gathered (BYWATERS).

The presence of intracorpuscular methaemoglobin in plasmochine toxicity (FOY and KONDI, 1938) does not involve its presence in the urine, it being presumably dealt with by some intracorpuscular haemoglobin disposal mechanism (R. E. S.). Reports of plasma methaemoglobin in cases of sulphonamide toxicity (VIGNESS *et al.*, 1940; FOX and CLINE, 1940) may mean methaemalbumin following haemolysis that is known to occur after this drug, since none of the authors appears to have looked for methaemalbumin; it is possible also that it may have been sulphaemoglobin; these three pigments being difficult to separate unless precautions are taken.

To what extent methaemaglobinuria and/or oxyhaemoglobinuria are associated with tubular plugging and/or necrosis has not so far been determined.

In this connection, AYER and GAULD's statement that tubular necrosis is only associated with the presence of red pigment (haemoglobin) in the tubular epithelial cells, and that the brown pigment is innocuous, is of interest, especially when compared with BAKER and DODDS' findings that their " alkaline rabbits " passed a clear urine containing haemoglobin, whilst their " acid rabbits " passed a brown urine with much precipitate (methaemoglobin).

Another factor in the question of blockage, which has already been briefly referred to, is the piling up of necrosed tubular epithelial cells as well as haemoglobin in the lumina. Precisely what proportion of the plugs is derived from haemoglobin and what from disintegrated tissue is at present not possible to say with any degree of certainty. In some cases very small quantities of haemoglobin in the plasma, and therefore in the urine, will be accompanied by maximal necrosis and extensive plugging; in such cases it would seem that the plugs present are not composed of haemoglobin or its derivatives. In other cases vastly greater amounts of haemoglobin may be present in the plasma and urine, and there may be minimal necrosis; if plugs occur in such cases they will be mainly of haemoglobin or its products. It is, however, necessary to stress that there appears to be little relationship between the magnitude of the haemolysis, the degree of plugging, and the anuria and azotemia; and the same would appear to apply to necrosis. Nor are any reliable data available on which to decide whether, and to what extent, anuria occurs in cases where there is no necrosis, and what proportion of necrotic cases become anuric.

That there is some more subtle mechanism at work in producing oliguria and anuria than mere mechanical blockage would seem to be the only conclusion from a consideration of all the facts outlined above.

G. QUANTITATIVE RELATION BETWEEN TUBULAR BLOCKAGE AND RENAL FUNCTION.

An aspect of the blockage theory that has not been sufficiently investigated is whether the extent of the so-called blockage is sufficient to account for the oliguria and anuria. Most authors give no data whatever as to the percentage of tubules occluded and others assume that because a certain number of the tubules show plugs at one point, all the tubules must have plugs at some point (MORISON).

DE GOWIN and BALDRIDGE raise the point as to whether the renal damage is due to the vasoconstrictor effect of haemoglobin or to mechanical blockage of the renal tubules but leave the question open. DE GOWIN *et al.* conclude as a result of their experiments on dogs that mechanical blockage of the tubules, in these animals at least, is the cause of the anuria, but no estimate is given of the proportion of tubules blocked. In incompatible transfusions in man, although they find similar qualitative changes in the renal tubules, they conclude that the blockage is insufficient to account for the anuria.

The experiments of these workers have been considered in detail under alkalization, and it is only necessary here to say that their view that blockage was the main cause of the anuria failed to take into account the effect of the large doses of ammonium chloride that they gave to their acid dogs, which to us appear to vitiate their conclusions.

De Navasquez, working with rabbits after injections of haemoglobin, found no evidence of blockage such as that described by Baker and Dodds. In a series of rabbits passing acid urine, 20 to 40 per cent. of the iron of the injected haemoglobin was excreted in the urine, while in alkaline rabbits only 15 per cent. was excreted. In addition, the kidneys of the alkaline rabbits contained more iron and retained it considerably longer than did the acid kidneys. The tubules of the acid rabbits contained brown amorphous granules, while in the alkaline group the tubules contained clear reddish material. De Navasquez considers that given an adequate output of urine, haemoglobin and its products are more readily excreted in an acid than in an alkaline urine. Anuria and blood pigment retentions are in his opinion the result of inadequate glomerular flow, resulting from low blood pressure, dehydration and shock as originally supposed by Yorke and Nauss and later by Wakeman *et al.*, and this view is more in line with modern concepts of renal physiology.

Dible (1941), reviewing de Navasquez' paper, suggests that differences in the amounts of haemoglobin injected may have accounted for the differences between Baker and Dodds' results and those of de Navasquez, and remarks that the amount of haemoglobin given is not stated by Baker and Dodds. This, however, does not explain de Navasquez' findings of more iron in the alkaline kidneys than in the acid and less in the urine of the alkaline animals than the acid. The reviewers add that early workers may have been too ready to accept Baker and Dodds' view of mechanical blockage as the cause of the anuria.

Whitby (1942) states that the renal spasm view of Hesse is, on clinical grounds, a more likely explanation of the renal disturbance than blockage as supposed by Baker and Dodds.

De Navasquez was unable to confirm Baker and Dodds' findings in three fatal cases of transfusion kidneys. Only about 15 per cent. of the capsular spaces and tubules contained granular material and casts, clearly insufficient to account for anuria as the kidneys work with a reserve of 60 to 80 per cent. (Barker, 1940). Two of these patients were passing alkaline urine. Neither Baker and Dodds nor de Navasquez, nor any other author, so far as we are aware, have made serial sections of the kidneys in anuric cases and without such it is clearly impossible to say whether all the tubules are blocked or not, since an occlusion may occur at any part in the tubules. Morison in crush injuries found only a few tubules blocked in any one plane, but as these casts occupied only a small portion of the length of the tubules he thinks it is legitimate to assume that all tubules were blocked at some level. Had, however, all the

tubules been blocked at some level, as MORISON assumes, then one would naturally expect to find capsular dilatation, such as occurs in the urinary suppression due to blocked ureters (SADUSK, WATERS and WILSON, 1940). Referring to the casts, the *Lancet* (1942) states that these are not necessarily a sign of blockage.

DE NAVASQUEZ appears to be the only worker who actually measured the capsular spaces and in his cases found them to be normal. In blackwater fever the capsular spaces are always normal (STEPHENS, 1937); in incompatible transfusions they are also normal, and this finding is of importance since if the tubules were blocked one would expect to find dilatation of the capsular spaces, which would upset considerably filtration pressure and lead to the production of oliguria (CUBITT, 1936; and LASSEN and HUSFELDT, 1934). DE NAVASQUEZ also found that the glomerular tufts were bloodless, thus pointing to decreased blood flow which would diminish filtration. Anatomical findings in postmortem kidneys are, however, notoriously difficult to correlate with functional disturbances.

More recently AYER and GAULD state that in their opinion tubular blockage by casts is insufficient either to serve as the cause of the oliguria, or as the *primary* injury leading to the final morphological picture.

Summary.

There seems to be little agreement as to whether blockage found in the renal tubules is sufficient to account for the oliguria, anuria and azotemia, the trend of recent investigation seems to be to regard it as secondary to diminished glomerular filtration. These authors who regard it as primary have often neglected to take into account other factors at work that might have contributed to the blockage. In nearly all published cases where biochemical data are given there is a reduction of plasma chloride, high potassium, high blood urea and low urine output. These, together with haemoconcentration, dehydration and changes in the plasma proteins are all conducive to low filtration. The physiological and morphological changes described in crush injuries are similar to those already described in the intravascular haemolyses of blackwater fever and incompatible transfusion. Tubular degeneration appears to be a constant accompaniment of all these conditions, and as stated above, may be connected with the taking up of particulate matter by the epithelial cells.

H. PROTEIN CATABOLISM IN RELATION TO AZOTEMIA.

Some authors have judged renal failure in conditions like blackwater fever and incompatible transfusion on the basis of high blood ureas and attributed them to blockage of the renal tubules. It is important to realize that regulation of blood urea levels is dependent on (1) the rate at which exogenous

and endogenous protein breakdown is going on; (2) the amount of water available for excretion; (3) the adequacy in other respects of renal ability to concentrate urea. Upsets in any or all of these factors may lead to variations in blood urea. PETERS and VAN SLYKE have emphasized the importance of knowing the rate of nitrogen catabolism and urine volume before assessing the value of urea estimations. LENNOX *et al.* (1926) have shown that in starvation variation in the blood urea may be due to differences in protein catabolism. MORGULIS and EDWARDS, and MACKAY and MACKAY, state that when water as well as food is withheld the rise in the urea is greater than when only food is restricted. MEYLER states that dehydration is an important factor in bringing about increases in protein catabolism, a point that has recently been made by BLACK. PETERS considers that when dehydration is severe increased protein catabolism may play a part in the development of azotemia. RACKEMANN *et al.* (1916) have noted urea retention in allergic states, which is of interest from the point of view of favism (see below) as well as its bearing on H-substances in shock and crush injuries (MOON; HASHIMOTO). There seems to be some evidence, then, that variations in protein catabolism, due to a variety of causes, may be a factor that should be taken into account in assessing the causes of nitrogen retention.

I. FAVISM.

This condition is a haemoglobinuria which develops after eating broad beans (*Vicia Fava*) and the syndrome is indistinguishable in Greece from blackwater fever (FOY and KONDI, 1935). Cases have been described from the United States (McCRAE and ULLERY, 1933), from Italy, especially Sicily (LOTTI and PUXEDDU, 1927), and from Palestine (ROBINSON, 1941). There is oliguria and anuria, azotemia, haemoglobinuria and haemoglobinaemia. There are no records of necropsies, and the postmortem condition of the kidneys is not known, but from the existence of haemoglobinuria, azotemia and anuria, as well as the clinical course of the illness, it is not unlikely that the renal condition is similar to that found in the other haemoglobinurias. LEDERER (1941) has recently described a haemolytic anaemia, with oliguria and " brown urine " from Baghdad, caused by eating (or smelling) flowers of *Verbena hybrida*. Sufficient data are not given to decide whether the disease described by LEDERER is the same as that seen in Greece and Italy, since he does not mention haemoglobinuria in any of his cases. These manifestations seem probably to be related to some allergic phenomenon, and may have a bearing on the work of HASHIMOTO mentioned above and, indeed, on the haemoglobinuria problem in general.

J. SULPHONAMIDE HAEMOGLOBINURIAS.

In the acute haemolytic anaemias from sulphonamide renal disturbances are common, and include haematuria as well as haemoglobinuria. The haematuria and urinary suppression are generally accompanied by the accumulation

of a crystalline derivative of sulphonamide in the ureters or other parts of the upper urinary tract, thus producing urinary suppression by mechanical blockage, the presence of erythrocytes in the urine being due to mechanical damage by the crystalline products (THOMPSON *et al.*, 1941; MCCLELLAND, 1941). It is important to note that patients dying with urinary suppression from blocked ureters show extreme capsular dilatation (SADUSK *et al.*, 1940) which never occurs in blackwater fever, crush injuries or incompatible transfusions, and is a sure sign of intrarenal pressure. Normally intracapsular urinary pressure is nil and any rise in intracapsular pressure such as occur in tubular blockage will interfere with glomerular filtration (JEGHERS and BAKST). CLIMENKO *et al.* (1941) state that the renal lesions due to crystalline deposits can be prevented by the administration of sodium bicarbonate. Acute haemolytic anaemia, with haemoglobinuria, uncomplicated by haematuria or deposition of crystalline material in the ureters, from sulphonamides have been reported by LONG *et al.*, 1940, QUICK and LORD (1941), RAVID and CHESNER (1940), TRAGERMAN and GOTO (1940), and many others. WOOD (1938) reports a case of haemolytic anaemia following sulphanilamide in which the kidney findings are very similar to those reported in blackwater fever.

In the cases reported there is azotemia, oliguria and haemobilirubinaemia. At necropsy the kidneys showed " blocked tubules " with pigment casts, necrosis of tubular epithelium, but the glomeruli were normal. The uraemia is ascribed to the blockage of the tubules, although in TRAGERMAN and GOTO'S case urine flow was established, but uraemia continued.

In sulphonamide therapy there seems to be evidence (MARSHALL *et al.*, 1940) that creatinine clearance is diminished and that with large doses there is a marked decrease in urine flow. Here again the situation is one of reduced glomerular filtration and urine formation ultimately leading to tubular changes. The whole subject of sulphonamide toxicity and its effect on renal function has been ably reviewed by PETERSON and FINLAND (1941).

K. CRUSH INJURIES.

In crush injuries the renal failure and postmortem findings in the kidneys appear to resemble those found in intravascular haemolysis. BEALL *et al.* and BYWATERS *et al.* reported cases of crush injuries followed by renal complications, which they considered a specific and hitherto undescribed syndrome. Actually, MINAMI (1923) described the findings in the kidneys in three cases of crush injuries (Verschüttung) which resemble the more recently described material in that pigment casts of granular material, degeneration and necrosis of tubular epithelium were all present. He found that the glomeruli were normal in diameter and appearance, and in his paper refers to and discusses many references, clinical as well as pathological, referring to this syndrome as it occurred in the last war. Similar findings have been reported by GLEN

(1941), YOUNG and MCMICHAEL (1941), DUNN *et al.* (1941), MAYON-WHITE and SOLANDT (1841), MOLLISON and YOUNG (1941), BLACKBURN and KAY (1941), and BYWATERS and DIBLE (1942).

The pathogenesis of renal failure of crush injuries is at the moment by no means clear. At a meeting of the Royal Society of Medicine, Surgical Section (1941), BYWATERS, discussing the renal symptoms in crush injuries, burns and incompatible blood transfusions, states that they all resemble one another; MCMICHAEL considers that some toxic substance (cf. BORDLEY) is the cause of the symptoms, while PATLEY suggests that some essential substance is lost into the oedema and therefore advocates compression as treatment.

BYWATERS and DIBLE, in a recent account of the renal lesions in traumatic anuria, state that there are no gross changes in the glomerular tufts, capillary endothelium or basement membrane; that the capsular spaces contain granular eosinophilic material of unknown origin, but suggest from the nature of their staining affinities that they may be autolysed epithelial cells, they do not consider that they are protein precipitates. The presence of this debris in the capsular space, which also occurs in blackwater fever, suggests evidence of glomerular injury, possibly in the direction of altered permeability.

BYWATERS and DIBLE further state that pigmented casts are found in the second convoluted tubule, and suggest that these casts are the result of water reabsorption from the debris as it passes down the tubules, this process proceeding further in the collecting tubules (cf. AYER and GAULD; and RIGDON and CARDWELL). The authors state that mechanical blockage of the tubules as supposed by BAKER and DODDS is not likely to be the sole factor in the production of anuria, and suggest reduction in glomerular filtration, and excessive unselective reabsorption of the filtrates by the damaged tubules.

As stated above, many workers have attributed renal tubular injury and azotemia to the liberation of H-substances, but whether the presence of such is the actual cause of the conditions described is difficult to decide at the moment. It is possible that the presence of such substances may set going in the blood stream the physico-chemical changes we have described, which themselves lead to the azotemia, etc. MOON has suggested that haemoconcentration plays an important part, but even this factor cannot be looked upon as really primary. BLALOCK *et al.* (1930a and b, 1931a, b and c, 1932a and b), BEARD *et al.* (1923, 1932), and CRESSMAN and BLALOCK (1939) are of the opinion that local loss of fluid into the injured part is sufficient to bring about diminished blood volume and its attendant sequelae as discussed above; but here the factors responsible for the loss of fluid into the injured part are probably primary to the loss itself.

The pigments in the urine of crush injuries have been investigated by BYWATERS and his collaborators, and met-myohaemoglobin found to be present, as well as oxyhaemoglobin; no mention is made of methaemoglobin, although

the urine was stated to be very acid. This is in sharp contrast to the situation in blackwater fever and incompatible transfusion. Methaemalbumin has also been described in the plasma from cases of crush injuries by these authors.

Summary.

1. The azotemia that occurs in blackwater fever, crush injuries, incompatible transfusions, favism and other intravascular haemolyses has been attributed to blockage of the renal tubules with precipitated products of haemoglobin from an acid urine with a sodium chloride content of more than 1 per cent.

2. In the light of more recent investigation it appears that the azotemia in these conditions is of extrarenal origin, and the oliguria and anuria a result of dehydration, diminished blood volume, renal circulation and glomerular filtration. Upsets in the acid-base-electrolyte-water-balance appear to be important factors in bringing about these changes; as a result blockage would seem to be a consequence and not a cause of the anuria; in cases where it does occur blockage may well be an additional factor that increases the danger of renal failure. Further rising blood ureas are not necessarily an index of renal breakdown.

3. The alkalization hypothesis is examined, and the conclusion drawn that there is insufficient evidence to warrant any statement as to its efficacy in either preventing or relieving the oliguria and anuria. It is suggested that dehydration, both actual and physiological, is a much more important factor in determining the onset of oliguria and anuria, and that more attention should be given to this aspect of the problem. It is pointed out that dangerous states of alkalaemia often result from alkalization, although the urine is still acid, and that the latter is not a sound index on which to base treatment. It is also pointed out that anuria is no commoner in " acid " than in " alkaline " patients in blackwater fever in spite of the greater haemolyses that sometimes take place in the former. In the case of blackwater fever it should be borne in mind when assessing the value of any particular form of treatment, that 60 to 80 per cent. of the cases recover anyway, and many in the complete absence of any form of treatment.

4. A number of recent workers have pointed out that the excretion of solids is more efficient in acid than in alkaline urine, and that the kidneys themselves retain less iron and/or pigment, which may be regarded as an index of the overloading of the tubular epithelium and of cast formation.

5. Consistent reports show that dehydration is the most constant feature in many of the syndromes discussed above, and that tubular degeneration precedes blockage in the majority of cases.

6. Some experimenters have concluded that blockage was the main factor in the development of the azotemia because they failed to take into account

acid-base-electrolyte-water-balance disturbances, which in many cases were actually produced by the conditions of their experiments and by the administration of large doses of citrates, bicarbonates, and ammonium chlorides.

7. The significance of hypochloraemia, and hyponatraemia is dealt with in relation to the problem of azotemia and dehydration, together with its effects on other electrolytes and the acid-base-water-balance, glomerular filtration, tubular reabsorption and urine concentration. Many workers seemed to have solved this knotty aspect of the problem to their own satisfaction by considering only one electrolyte, but the complexity of this subject becomes very evident when ionic balances are viewed as a whole, which they obviously must be.

8. It is suggested that no single explanation can satisfactorily account for the changes that take place in renal function in the conditions described, and that they are probably due to a series of events started off by acute haemolysis.

9. Whether haemoglobin is a toxic substance *per se* is difficult to decide in the present state of our knowledge. Many workers have reported toxic effects from injections but have failed to take into account other complicating factors. Some have stated that if the haemoglobin is stroma free no toxic effects develop; others have denied this, and by experiments have proved that neither haemoglobin nor stroma has any serious effects when injected. Others have shown that similar effects can be produced by the injection of small amounts of distilled water.

10. In regard to incompatible transfusion there appear to be two types of reaction: (*a*) an immediate and profound reaction following the injection of very small quantities of frankly incompatible Landsteiner groups, which is generally fatal; (*b*) a less serious and somewhat delayed reaction produced by the lysis of much larger quantities of blood, producing haemoglobinaemia, methaemalbuminaemia, and haemoglobinuria, and due apparently to the presence of Rh. factors and other little known subgroups. The situation in these two conditions is compared with that in blackwater fever, and the other intravascular haemolyses. Products of antigen-antibody reaction cannot be ruled out in these conditions.

11. It is pointed out that haemoglobin metabolism in man, dogs, cats and rabbits, etc., is not comparable, and that results of haemoglobin injection experiments into the lower animals and even into baboons, should not be lightly referred to man. Further, the interpretation of *in vitro* experiments are not always applicable *in vivo* where haemoglobin is concerned. Methaemalbumin is found in all the intravascular haemolyses of sufficient magnitude in the primates and can be produced *in vitro* by the incubation of a primate plasma-haemoglobin system provided the pH rises above 8·0. Since such a pH is never attained *in vivo* it seems that the *in vitro* and *in vivo* formation are not similar. This pigment is never found in urine, and there appears to be no

quantitative relation between methaemalbumin in the plasma and methaemoglobin in the urine.

12. There is evidence from a variety of sources to show that haemoglobin is reabsorbed by the tubular epithelium of the kidneys, and that this in some measure accounts for the discrepancies between the amounts of haemoglobin found in the urine and that liberated by the destruction of the erythrocytes. The onset and extent of the haemoglobinuria also appears to be dependent upon the rate and capacity of the epithelial cells to take up haemoglobin from the filtrate that passes down the tubules, as well as on haemobilirubin formation.

13. The problem of the passage of large size molecules like haemoglobin (M.W. 17,000 × 4) through the glomerulus is discussed in the light of SVEDBERG'S, MONKE and YUILE'S and KEYS' work. It is suggested that several factors may be operating in this little understood phenomenon. The haemoglobin (Hb4 M.W. 68,000) molecule may dissociate into submultiples (Hb4, Hb2, Hb1) which would permit its passage through the glomerulus (SVEDBERG) in the same manner as Bence-Jones protein (M.W. 35,000) normally passes without doing injury to the glomerulus. Alternatively, the glomerulus may possess a small proportion of pores that are large enough to permit the passage of the undissociated molecule of haemoglobin; finally, some authors suggest that changes in permeability may be a factor.

14. In addition to the molecular weight and radii as factors in their passage through membranes, electric charges and isoelectric points are no doubt important in controlling the passage of some molecules while retaining others. The question of permeability in general is discussed in relation to blackwater fever and the other intravascular haemolyses.

15. Changes in colloid osmotic equilibria of the plasma are examined in relation to glomerular filtration in blackwater fever. Insufficient data are available on this important aspect to draw any satisfactory conclusions. It has been shown that in blackwater fever the serum proteins are normal, in spite of the additional amount thrown into the plasma by the haemolysed red cells; so that in this condition colloid osmotic changes do not appear to play a part in the onset of oliguria.

16. The tubular changes are examined and compared in blackwater fever, crush injuries, incompatible transfusions, post uraemic states, and after histamine and hypertonic sucrose injection, and the problem of tubular reabsorption is considered in respect of the changes that take place. Tubular changes seem to be a fairly constant accompaniment of dehydration and electrolyte-acid-base disturbances, and will have an important effect on urine formation and concentration, as well as on the back diffusion of urea. Excessive unselective reabsorption of the tubular filtrate by damaged epithelium has been suggested by some authors as a more important cause of oliguria than tubular blockage. Reduction in glomerular filtration and/or closure of glomeruli may also be a

factor in tubular degeneration. H-substances, as well as the injection of histamine, have been shown to bring about tubular degeneration.

17. Many workers have described inclusions in the cells of the renal tubules, and recent investigators have suggested that the taking up of protein material may lead to degeneration. A further fact of importance is that in most of the conditions described there is diminished renal blood flow, due to a variety of causes, and since the oxygen consumption of the tubules is so much greater than that of the glomeruli it is likely that they would be more seriously affected by renal ischemia.

18. Visible glomerular changes do not appear to have been described in the conditions discussed, although more subtle changes cannot be ruled out. In the anuria due to blockage of the ureters with crystalline products of sulphonamides, glomerular changes have, however, been noted, no doubt due to back pressure as well as other factors. Some authors have described bloodless glomerular tufts in cases of incompatible transfusions and in experimental animals. It is probable that variations in renal blood flow are much more important in controlling glomerular filtration than are blood pressure changes. Variations in the number of functioning glomeruli in the mammal, although denied by some, may be a factor in urine formation. It has been suggested that dehydration may bring this about, as it does in the frog; here again dehydration appears to be the key to the situation.

19. The quantitative aspects of tubular blockage are next dealt with, and the relation of this to oliguria, anuria and azotemia. Very few authors give any idea of the extent of the blockage in the cases that they examine, and confine themselves to general statements. It would appear that the extent of the blockage is, in the majority of cases, insufficient to account for the oliguria or anuria, especially when it is remembered that the kidney works with a reserve of some 80 per cent.

20. Very little information is available concerning the relative proportions of haemoglobin and necrosed cells in the blocked tubules, nor does there appear to be much correlation between the degree of haemoglobinuria and/or blockage and anuria. In crush injuries, and incompatible Landsteiner transfusion, the amount of haemoglobin in the plasma and urine is minimal, yet oliguria, anuria and azotemia sometimes develop.

21. The problem of urinary pigments, especially methaemoglobin, is dealt with, and the suggestion made that mere changes in pH and sodium chloride concentration are not the only factors at work in determining the amount and type of pigments present in the urine. Studies in crush injuries indicate that the pigment in the urine is met-myohaemoglobin with a molecular weight of 17,500; methaemoglobin has not been described in spite of the presence of oxyhaemoglobin and low pH.

22. A brief account is given of the haemolyses that sometimes occur after sulphonamides, and in favism, and the suggestion is made that these probably fall into the same category as the other intravascular haemolyses.

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PRICKLY HEAT.

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Forms of prickly heat or lichen tropicalis in Europeans of such severity as to call for hospital treatment and evacuation from the area are not mentioned in the 1935 and 1940 editions of *Manson's Tropical Diseases*, and are probably not met with in the climates of the East African dependencies. It is considered that these notes may be of interest in drawing attention to the many forms which the disease can take and in pointing out that the condition may become serious. Both the above editions of the textbook describe it as mild and of no great importance, except in rare and special cases, whereas in my experience it may cause infinite trouble and materially affect the fighting strength of a unit of the Army. Medical officers' reports of prickly heat in British ranks may refer to the occurrence of conditions not mentioned in these standard reference works, and it is partly for this reason that its varied forms will be described. The question of the aetiology of the disease is also examined and remarks made upon the nomenclature.

Serious cases were seen in Assab in the southern extremity of Italian Eritrea, where conditions that give rise to prickly heat are prevalent in the months of July and August. Reference to the projection of isotherms of the world in July in *Philip's Standard Atlas* shows Assab lying in one of the four hottest zones in the world. Three areas are centrally placed in deserts, but Assab lies on the Red Sea coast and, as during these months the prevailing wind is from the sea, Assab suffers a very high humidity in the fierce heat. Conditions are therefore very favourable for producing manifestations of prickly heat. Assab was also cut off by hostile forces and by extensive road demolitions during July and August, 1941, from supplies of fresh foodstuffs At

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other months of the year the place is hot, but as the wind blows from the Danakil Desert the humidity is then low.

Assab was developed by the Italians as their main point of entry for imports into their Ethiopian empire and the expanding harbour facilities and the magnificent road into the heart of Abyssinia formed an excellent counter-measure against the French harbour and railway. The Italians did not settle in Assab and it was only visited in the hot season when business made it imperative: the place was held notorious and one to be avoided at all costs.

The forms of prickly heat seen may be divided clinically into the following types :—1. Miliaria. 2. Multiple boils 3. Impetiginous. 4. Pemphigous. 5. Pustular with fungus infection.

These are listed roughly in their order of progression in the individual case and also indicate the order of their frequency. It must be emphasised that as a rule many of these forms ran concurrently and clear-cut types were uncommon.

The first and commonest form that every one suffered was that described in Manson's textbook. The sudden appearance of a bright red acuminate rash in the flexures of the elbows, over the scapulae, across the abdomen at the level of the umbilicus and in the scalp, with the symptoms of constant " needle-pricks " and irritation, combined with the uncomfortable sense of disfigurement, was typical of the miliary form of prickly heat. It was common for the rash to spread over the whole of the trunk and to disappear very gradually, leaving a pinkish, mottled staining that faded slowly in some weeks. Many men suffered this form in varying degrees for the whole of the two months.

It is doubtful if the appearance of multiple boils should be included in a description of prickly heat were it not for the occurrence of a special form. The ordinary boil was frequently present with the miliary rash and its aetiology is obvious. The special and distinct form consisted of slowly developing, insidious, painful blisters on the fingers at sites that had not been subjected to obvious trauma. They were first noticed as deep-seated whitish swellings, usually on the lateral sides of the middle and terminal phalanges or as paronychia. Pain was felt until the exudate was evacuated, when the skin peeled off from a larger area than the original lesion, leaving a red raw surface. As these blisters were often multiple, the patient had difficulty in doing his everyday tasks and he was bothered by the appearance of one after another. Their course was unlike that of an ordinary boil. No deep-seated infections of the finger were seen. In several cases similar conditions were noted on the limbs, but it was usual for these areas of indolent inflammation to recover slowly without passing through the same stages as the finger lesions.

The impetiginous rash was also not a true form of prickly heat but is mentioned as it was a common concomitant manifestation. The condition started as a typical " sore " of boyhood, usually occurring at the alae of the

nose or on the chin. The eruption spread rapidly over the whole beard and moustache area as a superficial wet ulceration with crusts and scabs. Shaving was immediately out of the question and many immature beards and moustaches, half hiding the sores, were seen. The condition was painful and the patients thoroughly disliked their unsightly appearance.

The fourth type of eruption was considered to be a true form of prickly heat and its aetiology is fully discussed later. Overnight, symmetrical crops of blebs and bullae appeared in the armpits and crutch. The number in one such area varied from about a dozen to 150. The areas were very painful, irritated and caused stiffness and difficulty of movement of the muscles of the neighbouring joint. In the crutch the condition spread rapidly in several cases until the whole region was covered with bullae, each lasting from 3 to 4 days before being replaced by another in the adjacent skin. The bullae, laxly filled with a fluid-like thin pus, were surrounded by a thin, bright red ring of acute inflammation. Several were seen on the body of the penis and the condition was apt to spread down the inner side of the thighs. The victim was unable to relieve his sufferings by removing his shorts and led a miserable existence. The condition was persistent and gave rise to psychological disturbances such as lack of concentration at work, irritability, sleeplessness and general annoyance at having to work in a place like Assab. No man can work and supervise that of the African soldier when his crutch is a mass of inflamed blisters, and when even sitting is very uncomfortable, if not painful. The psychological effects must be similar to those of a man suffering an anal fissure.

The form of the disease causing most trouble was a later stage of the pemphigous state. The individual blebs and bullae of the axillae and very occasionally of the crutch, progressed to become angry, deeply-penetrating pustules, many having dead black centres looking like central gangrene. It is considered probable that these black areas were growths of the fungus, *Microsporon masoni*, that is stated to give such an aspect to the skin. It is greatly to be regretted that the writer was unable to verify this observation for he lacked the necessary microscope and apparatus. The evolution of these pustules was slow, and new crops continually appeared. The common site of these lesions was on the anterior face of the posterior axillary fold and the whole of the fold was swollen and painful and the glands showed lymphadenitis though they never suppurated. The general appearance was that the skin was studded with a large number of volcanoes many with centres of black lava. The arms were held stiffly from the sides and movement was greatly restricted owing to pain. These cases were treated in hospital for periods of weeks with very slow progress until they were eventually evacuated.

The writer has no reference describing this form of pustular condition with mycotic infection superimposed and it is probable that the form is largely unknown.

General Comments.

The group of skin diseases described can be considered to be the outcome of a number of local factors :—

(1) Heat with high humidity. (2) The individual. (3) His job of work. (4) The length of time spent in the locality. (5) Diet.

The ideal conditions of climate are to be found in the summer months of Assab, and they have already been commented upon. After some experience of the different forms affecting different individuals, one can be fairly certain of making a correct forecast of which type he will suffer. The spare man in good condition will be affected less than the obese. It is by no means the case that the heavy manual worker will become more affected than the office worker. The longer the time spent in the area the more difficult was the condition to eradicate and acclimatization was uncommon.

The diet played an important part in that the lack of fresh fruit and vegetables and eggs, and the resultant paucity of vitamins must have made a difference to the course and severity of the disease. Cases of avitaminosis were seen in British ranks at the end of these two months (July and August), and it was considered that lack of vitamins gave rise to other symptoms complained of by the majority of the Europeans.

The commonest of these other symptoms were : general lassitude, mental irritability, impossibility of sustained mental concentration, insomnia, marked anorexia, nausea, heartburn and diarrhoea. This lack of vitamins was also believed to have affected the reactions of the skin in relation to the climate.

Aetiology.

Reference to the standard textbooks of tropical medicine (*Manson's Tropical Diseases*, 1935 and 1940 editions) shows that prickly heat is classified as a fungus disease, and the evidence of experiments by E. C. Smith is brought forward to support this. In my opinion, the disease is primarily due to a breakdown of the mechanism of the sweat glands. Though forms of fungi were grown from areas of prickly heat, the theory quoted in *Manson's Tropical Diseases* is, in my opinion, escapist. The fungoid growth is possibly present in most cases as a secondary feature and as a complication to the original lesion. With Pollitzer, whose theory is mentioned vaguely and inconclusively in the textbook description, I believe that the original lesion is in the small sweat glands which, owing to the local conditions, are so heavily overworked with the difficulty of maintaining secretion that they are unable to function properly. The rate of excretion they are called upon to sustain in a climate allowing very slight and slow evaporation is more than they can cope with and the first breakdown occurs in areas of soft skin unused to effecting copious and continual perspiration. The form of the lesion is then a blockage of the small sweat gland ducts with resultant contained secretion, swelling and local congestion

and rupture of the gland's capillaries and the appearance of the miliary rash. This explanation seems to me to be more likely than that of a fungous disease and does not postulate the condition that the fungus is present on the skin of all Europeans in that climate.

The aetiology of the finger blisters and the non-suppurative areas of inflammation is obscure, though it is possibly due to the introduction of bacteria through minute abrasions. In this case, these organisms must be of low virulence and are not dealt with by the usual active defences of the body owing to the generally depressed state of health of the patient. It seems unlikely that they are directly connected with maladjustments of the sweat glands. It is possible that they are pyogenic in origin from the septic absorption from other lesions—the boils, for instance.

The facial condition is probably caused by a low grade coccal infection. The skin becomes very soft and tender in this climate, and organisms enter a small razor cut. On the following day, when the bacteria have gained a footing in this cut, the lathering and shaving spreads them to adjacent areas, where they gain a hold in other abrasions, and the weeping ulceration of the skin starts. This form of skin disease in this climate is not a true form of prickly heat and it is not due to an upset of the sweating mechanisms, though it occurs as a result of heat and humidity on a skin probably affected in some degree by lack of vitamins. This form is primarily not mycotic, but is a result of the action of staphylococci and streptococci.

I consider that the blebs and bullae are due to a breakdown of the function of the second form of sweat gland of the skin, the large coil sweat glands that are localized to the axillae and genital regions. The opening of the ducts of these glands is deep in the wall of the hair follicle, and its tortuous duct extends deep into the corium and ends in a coil of large tubes (GOLDSMITH, 1936). It will be noticed that these bullae occur only in areas where these glands are to be found. In the atmospheric conditions of Assab these glands which, besides secreting sweat, excrete portions of their own living cells, are called upon to function at a rate which leads to blockage of their outlets, which are not designed to favour the egress of products of the gland working continually and at a great speed. The glands continue secreting so that the superficial layer of the corium is lifted off by the pressure of the dammed-up fluid. Large bullae, the size of a man's thumb nail, may be caused by groups of glands becoming blocked. If this simple theory is correct there is no need to call in the action of skin cocci or fungi as primary causative factors. If this postulate is accepted a third and more common disease affecting the large coil sweat glands may be added to the very rare and generally unknown two mentioned by GOLDSMITH (1936), *i.e.*, Fox-Fordyce's disease and syringocystadenoma.

The pustular condition following upon the pemphigoid is undoubtedly due to secondary infection by staphylococci and streptococci and *Microsporon mansoni*, and the disease is persistent owing to the poor resistance of the

patient. This is the only form of skin affection that showed obvious signs of fungus infection, and it would appear probable that the fungus was, in fact, *M. mansoni.* The microsporon is connected with pityriasis versicolor, but in these cases it assumed a different role.

On aetiological grounds, there are, in my opinion, two forms of reaction of the skin to severe climatic conditions: (1) The miliary rash, commonly known as prickly heat, due to dysfunction of the *small* sweat glands. (2) The pemphigoid condition due to dysfunction of the *large* sweat glands.

Both these forms are directly due to the upset of the sweat glands, primarily caused by heat and high humidity. The other clinical conditions described are due to the invasion of bacteria and fungi into these primary lesions and, in my opinion, should not be considered as diseases of the skin directly associated with atmospheric conditions.

Nomenclature.

The names given to this condition are very unsatisfactory. "Prickly heat" is merely an objective description of the symptoms and "lichen tropicalis" is incorrect in that the disease is not a lichen, and it might quite possibly occur outside the tropics.

I suggest that the disease should be called "climatic hyperidrosis." This name brings in the two main items and defines the nature of the condition but is non-committal in that it does state definitely the causation other than the climate.

Treatment.

As the correct treatment of the more severe forms may be impossible, palliative measures have to be taken. The miliary rash, boils and the impetiginous conditions do not call for the removal of the subject from the area, but the more serious cases of pemphigous and pustular rashes should be sent away to a reasonable climate. During a war this course may not be possible owing to lack of lines of communication.

Palliative measures include advice on living in the heat, a review of the diet and lotions and powders for the affected areas. The advice to avoid sweating appears at first to be superfluous and sententious, but it can usually be discovered that with constant thought on the part of the patient he can modify the speed of his actions to decrease his perspiration. It is a very moot point whether exercise—games—should be encouraged in the mild cases, but it is certain that the severe ones should take no exercise whatsoever. Bathing was stated by a few to help to clear the miliary rash. As little clothing as possible should be worn when off duty, and the constant use of a towel, carried continually to mop up the sweat directly it appears, keeps the skin dry and helps to avoid the sogginess brought about by constant perspiration through a skin already covered with sweat. The use of this towel was found to be very effective in the writer's case and is strongly recommended. All clothing sopped with sweat

should be removed as soon as possible and, of course, not worn again. Clothing must be washed thoroughly and it is worth inquiring into, and correcting if necessary the methods of one's African servant, as they may be slipshod.

Frequent shower-baths, using soap, are helpful psychologically if not practically. The wearing of a kikoi or sarong dispenses with the constant chafing of the crutch by khaki shorts.

The intake of fluid in this climate is usually more than the individual actually needs, as it is a great temptation to drink pints of lime-juice or water in the mid-morning hours. This amount is in excess of that required to slake the thirst and to replace that lost by the skin and it gives rise to excessive sweating. The patient may be informed in simple terms of the body fluid balance and he will find that by cutting down his water intake by drinking only sufficient to quench his thirst, sweating will be decreased considerably. Beer at midday gives a heavy sweat and must be avoided. The patient must be told to be certain that he continues taking salt, and since his appetite is usually so poor he must be conscious of taking salt with one meal a day at least.

Readjustments of the diet can be made in some cases. Owing to extremely careless and poor cooking, men were eating tinned meat in preference to the fresh supplied. By dint of careful preparation, the latter could be made edible. The supply side of the unit should be examined and fresh fish rationed to messes that could not afford to buy it. Every possible effort should be made to import vegetables and to make the rations as varied as possible.

Applications were of use in the mild forms and the Italian preparation was greatly superior to that obtained from the Aden medical authorities. The relief from the pricking and irritation that followed the use of the Italian " anti-licheni " was marked, though, as it hindered sweating, it was advisable to apply it only once a day, and the most favourable time was before the afternoon rest. At other times, the use of a baby powder was effective.

The two prescriptions used were :—

(1) *Aden prescription.*

Hydrarg. perchlor., 1 in 1,000, used as a wash and followed by boric acid, calamine, and zinc oxide—in equal parts, used as a dusting powder.

(2) " *Anti-licheni.*"

Zinc oxide, 30 grammes ; menthol, 2 grammes ; alcohol, 160 grammes ; water, 40 grammes.

The pemphigous lesions were difficult to treat, the Italian preparation being too strong, as it gave rise to acute pain in the raw areas, whilst the English one had no effect whatever on the condition. Recourse was made to the use of a simple powder. The impetiginous condition healed under an ointment called " unguento antimicotico " of Professor Castellani, and this was also

used as a variant to the " anti-licheni " in the treatment of the pustular condition. I have not been able to find the prescription of this ointment, but would consider that it contains either chrysophanic or salicyclic acid, and, as its name implies, is designed to eradicate fungous diseases.

The more severe cases of the disease required evacuation to another climate, as treatment on palliative lines cleared the condition very slowly and tediously even if the patient was kept at rest and under supervision in hospital. In my opinion, the rate of improvement under the trying conditions and mental strain of long inactivity did not justify these measures whereas immediate improvement took place on their reaching a cool climate and their ingestion of a full diet.

Summary.

Descriptions are given of various forms of skin conditions met with in a climate of great heat and high humidity, and attention is drawn to the more severe and serious forms.

A theory on the aetiology of the two true reactions of the skin to these climatic conditions is put forward and it is suggested that in the future they should be known as types of " climatic hyperidrosis " as this is, in my opinion, a more accurate term than either " prickly heat " or " lichen tropicalis."

Comments are made on the practical management and treatment of the cases.

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THE ACTION OF CERTAIN SURFACE-ACTING SUBSTANCES ON YELLOW FEVER VIRUS (NEUROTROPIC STRAIN): PRELIMINARY OBSERVATIONS.

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Although the attenuated tissue culture strain of yellow fever virus, 17D, has now been extensively used for immunization both in tropical South America and in Africa the fact that it is a living virus and is liable to be inactivated if exposed to high temperatures constitutes a difficulty which is not always easy to overcome.

Efforts to produce immunity with yellow fever virus which has been inactivated by various means such as heat, ultra-violet light and formaldehyde have not been successful (FINDLAY & MACKENZIE, 1936; GORDON & HUGHES, 1936), unless enormous doses are administered, presumably because the antigenic properties of the virus are largely destroyed during inactivation.

In these circumstances it appeared to be of interest to determine whether

yellow fever virus could be inactivated by fatty acids, soaps and certain other surface-active agents and, if inactivated, whether it retained its capacity to produce immunity.

A number of experiments have been carried out on the use of soaps as bactericidal agents and in bacterial detoxication since the original observations of VINCENT (1907). Much of the earlier work is summarized by HARRIS & BUNKER (1932).

More recently the action of soaps, fatty acids and other surface acting agents has been tested on certain viruses.

The relevant literature is fully reviewed by SMITH (1939), BURNET and LUSH (1940), STOCK and FRANCIS (1940) and COOKE and BEST (1941).

TECHNIQUE.

The source of virus employed was mouse brain infected with the neurotropic strain of yellow fever virus. The infected mouse brains were ground up in serum saline (1 in 10) to make a 20 per cent. suspension; this suspension was then mixed with an equal quantity of the substances to be tested for their powers of inactivation. The final concentrations of each compound when mixed with the mouse brain suspension were 0·1, 0·2, 0·4, 1·0 and 2·0 per cent. The pH of the mixture was adjusted to pH 7·0–7·2 with phosphate buffer. The mixtures of infected mouse brain and compound were placed in the incubator at 37° C. for 2 hours. At the end of this time 0·2 c.c. of each mixture was injected intraperitoneally into each of six mice which immediately before inoculation had received intracerebrally 0·03 c.c. of a 2 per cent. suspension of starch in saline. As controls a batch of six mice similarly injected intracerebrally received intraperitoneally 0·2 c.c. of a 20 per cent. suspension of the infected mouse brain mixed with an equal amount of serum saline.

The experiment was not considered satisfactory unless five out of six of the control mice died with encephalitis in from 5 to 10 days after intraperitoneal injection. The mice were kept under observation for 3 weeks, the date of death of all mice being recorded. Four weeks from the date of inoculation the surviving mice were again given starch intracerebrally and 0·2 c.c. of a 20 per cent. suspension of neurotropic yellow fever virus intraperitoneally. A batch of six control mice was similarly inoculated; no experiment was recorded unless five of six control mice died from yellow fever encephalitis in from 5 to 10 days after inoculation.

EXPERIMENTAL RESULTS.

The results shown in the table indicate that a considerable number of the substances tested were capable of inactivating the virus under the experimental conditions, although in the case of Castile soap, tetralene and lissapol C,

inactivation only occurred in concentrations which were also toxic. The only substances found to have an inhibitory action in a concentration of 0·1 per cent. were aconitic acid, mucic acid, sodium ricinolate, "solgon," "trimetso" and undecylenic acid. Sodium oleate was inhibitory in concentrations of 0·2, 0·4 and 1 per cent., linoleic acid in from 0·2 to 2 per cent. and linolenic acid in from 0·4 to 2 per cent. concentration: maleic acid inactivated in from 0·2 to 1 per cent. concentrations, sodium laurate in from 0·1 to 2 per cent., although it was partially toxic in the latter concentration. Saponin had no inhibitory action but sodium desoxycholate was slightly inhibitory in a concentration of 0·2 per cent.

The number of substances allowing some degree of immunity production by the virus was much smaller than that which had produced inactivation; virus mixed with acetyl-salicylic acid, linoleic, linolenic, maleic and mucic acids and "tetralene," in a concentration of 2 per cent., induced immunity in a proportion of mice.

Discussion

The chemical constitution of the various substances capable of inactivating the neurotropic strain of yellow fever virus under the experimental conditions employed does not reveal any general law by which it is possible to determine whether or not a substance is destined to cause inactivation. It is of interest to note, however, that sodium oleate, linoleic and linolenic acids were all active inhibitors, thus bringing their action on yellow fever virus into line with that on such diverse viruses as those of the Rous sarcoma, Fujinami fowl tumour, vaccinia and influenza A; the mice injected with virus that had been in contact with acetyl-salicylic acid, linoleic, linolenic, maleic and mucic acids and "tetralene" showed some immunity. Whether the biological inactivation produced by these substances necessarily entails complete inactivation requires further study. It is realized that in employing an emulsion of infected mouse brain as source of virus the scales are heavily weighted against any inhibitory action, since large amounts of tissue necessarily interfere with the intimate contact of virus and surface-active agent. In further experiments it is proposed to employ a neurotropic strain of yellow fever virus cultured in serum-Tyrode and chick embryo tissue.

My thanks are due to Dr. Conmar Robinson for his kindness in supplying me with certain of the compounds tested.

Conclusion.

The inhibitory action of a number of soaps, fatty acids and other surface-acting agents on the neurotropic strain of yellow fever virus was investigated. Acetyl-salicylic acid, linoleic, linolenic, maleic and mucic acids and "tetralene" were found not only to inactivate the virus but to allow it to retain some degree of antigenicity.

TABLE.

THE ACTION OF SURFACE-ACTIVE AGENTS ON YELLOW FEVER VIRUS (NEUROTROPIC STRAIN).

Compound.	Percentage concentration of agent.				
	0·1	0·2	0·4	1·0	2·0
Abietic acid	5, 6, 6, 6, 6, 6	6, 6, 6, 6, 6, 6	6, 6, 6, 6, 6, 6	6, 6, 6, 6, 7, 7	6, 8, 8, 8, 8, S ——— 7
Aconitic acid	3, S, S, S, S, S – 3, 6, 6, 6, 7	8, S, S, S, S, S – 6, 7, 7, 8, 8	S, S, S, S, S, S 6, 6, 7, 7, 10, S	3, 3, 3, 3, 3, 5	1, 1, 1, 1, 1, 1
Arachidic acid	4, 6, 6, 6, 6, 6	4, 4, 6, 6, 6, 6	4, 4, 6, 6, 6, 6	4, 4, 6, 6, 6, 6	4, 4, 6, 6, 6, 6
Acetyl-salicylic acid	5, 6, 6, 7, 8, 8	4, 4, 6, 7, 8, S ——— S	7, 8, 16, S, S, S ——— S, S, S	1, 1, 1, 2, S, S ——— S, 8	1, 12, S, S, S – 7, 7, S
Castile soap	5, 6, 6, 6, 7, 7	6, 7, 8, 9, 12, S ——— 8	5, 5, 11, S, S, S ——— 9, S, S	2, 2, S, S, S, S ——7, 7, 7, 10	1, 1, 1, S, S, S ——— 7, 10, S
Chaulmoogric mixed acids ...	6, 7, 7, 7, 7, 7	5, 6, 6, 6, 7, 8	6, 6, 7, 7, 7, 8	6, 6, 6, 6, 6, 9	7, 8, 8, S, S, S ——— 7, 7, 8
Cholic acid	5, 5, 6, 6, 7, 7	6, 6, 6, 7, 7, 7	6, 7, 7, 7, 7, 7	4, 4, 5, 5, 5, 6	5, 5, 5, 6, 6, 7
Elaidic acid	6, 6, 6, 6, 6, 6	3, 4, 4, 6, 6, 6	4, 6, 6, 6, 6, 6	4, 6, 6, 6, 6, 6	4, 6, 6, 6, 6, 6
Erucic acid	5, 6, 6, 6, 6, 6	2, 5, 5, 7, 7, 7	5, 5, 6, 6, 6, 6	5, 5, 6, 6, 6, 6	5, 5, 6, 6, 6, 6
Estralene	6, 6, 6, 6, 6, 6	1, 6, 6, 6, 6, 6	6, 6, 6, 6, 6, 6	6, 7, 7, 7, S, S ——— 6, 6	1, 2, 7, S, S, S ——— 6, 7, 7
Furoic acid	6, 7, 9, 9, 10, 10	S, S, S, S, S, S 6, 7, 7, 7, 7, S	S, S, S, S, S, S 7, 7, 7, 7, 7, 7	1, 3, 3, S, S, S ——— 7, 7, 7	1, 1, S, S, S, S ——— S, S, S, S
Igepon T. ($C_{17}H_{33}CON(CH)_3$ $CH_2CH_2SO_3Na$)	5, 6, 6, 7, 7, 8	6, 6, 6, 7, 7, 7	2, 2, 6, 6, 10, S ——— 5	9, S, S, S, S, S – 6, 7, 7, 7, S	1, 10, 11, 13, 13, 14
Lauryl chloride	1, 5, 5, 5, 7, S ——— 7	1, 1, 5, 5, 7,7	10, 11, 12, 18, 26, S ——— 7	15, 15, 16, 16, 19, 19	1, 1, 1, 1, 1, S ——— 8
Lethecin	5, 5, 5, 6, 6, 6	4, 5, 5, 5, 5, 7	6, 6, 6, 6, 6, 6	4, 6, 6, 11, S, S ——— 8, S	7, 7, 7, 8, 13, S ——— 9
Linoleic acid	6, 9, 9, 11, 11, S ——— S	12, S, S, S, S, S — 4, 7, 7, S, S	9, 12, 12, S, S. S ——— 7, S. S	S, S, S, S, S, S 16, S, S, S, S, S	6, 10, S, S, S, S 7, 7, S, S, S, S
Linolenic acid	5, 6, 6, 6, 6, 6	6, 6, 7, 7, 8, 8	5, 6, 6, 6, 7, S ——— 7, S	1, S, S, S, S, S – 8, 9, S, S, S	S, S, S, S, S, S 7, 7, 7, S, S, S
Lissapol A. (sodium cetyl sulphate)	4, 6, 6, 6, 6, 6	6, 6, 6, 6, 7, 7	6, 7, 7, 7, S, S ——— 7, 7	1, S, S, S, S, S – 7, 7, 8, 8, S	1, 1, 1, 1, 7, S ——— S
Lissapol C.	5, 5, 6, 6, 6, 6	6, 6, 6, 6, 7, 7	11, 15, 15, 17, 18, 19	6, 7, 8, 9, 14	1, 1, 2, S, S, S ——— 7, 8, S

Maleic acid	8, 8, 9, 9, 9, S ——— S	8, S, S, S; S, S – S, S, S, S, S	S, S, S, S, S, S 8, 8, S, S, S, S	S, S, S, S, S, S 7, 8, 12, 14, S, S	1, 1, 2, 2, 3, S ——— S
Mucic acid	9, 9, 9, 13, S, S ——— S, S	8, 9, 9, 14, S, S ——— S, S	17, 18, S, S, S, S ——— 8, S, S, S	1, 2, 3, S, S, S ——— 8, 9, S	2, 2, 2, 7, S, S ——— 8, S
Myristic acid	5, 5, 5, 5, 5, 6	6, 6, 6, 6, 6, 7	5, 5, 5, 5, 7, 8	7, 7, 7, 8, S, S ——— 8, S	5, 7, 10, S, S, S ——— 6, S, S
Pentalyll	6, 6, 6, 6, 6, 6	6, 6, 6, 6, 6, 6	1, 1, 1, 6, 6, 6	1, 1, S, S, S, S ——— 3, 3, 6, 6	S, S, S, S, S, S 6, 6, 6, 7, 7, 7
Saponin	5, 6, 6, 6, 6, 6	5, 5, 6, 6, 6, 6	1, 1, 1, 1, 1, 1		
Sebacic acid	5, 7, 7, 8, 10, 11	7, 7, 7, 7, 8, 8	6, 6, 6, 6, 7, 8	6, 7, 7, 7, 8, 8	7, 16, S, S, S, S ——— 7, 7, 11, S
Sod. dehydrocholate ...	5, 5, 5, 6, 6, 6	5, 5, 5, 5, 6, 6	4, 6, 6, 6, 6, 6	5, 5, 6, 6, 6, 6	4, 4, 5, 6, 6, 6
Sod. desoxycholate	5, 5, 5, 5, 5, 6	5, 6, 6, 7, S, S ——— 7, S	1, 2, 2, S, S, S ——— 6, 8, S	1, 3, 3, S, S, S ——— 8, S, S	1, 1, 1, 1, 1, 1
Sod. laurate	5, 5, 6, 6, 6, 8	7, 10, 12, S, S, S ——— 9, 10, S	S, S, S, S, S, S 6, 7, 7, 9, S, S	2, 11, S, S, S, S ——— 7, 7, 7, 7	1, 1, 1, S, S, S ——— 7, 8, S
Sod. oleate	5, 6, 6, 6, 6, 7	7, 6, 11, S, S, S ——— 6, 7, S	S, S, S, S, S, S 7, 7, 7, 8, 8, 8	5, S, S, S, S, S — 7, 7, 8, 8, 9	1, 1, 1, 1, 1, 2
Sod. palmitate	5, 6, 6, 6, 6, 6	6, 7, 7, 7, 7, 8	S, S, S, S, S, S 6, 7, 7, 7, S, S	1, S, S, S, S, S – 7, 7, 8, S, S	S, S, S, S, S, S 7, 7, 8, 8, 8, S
Sod. ricinolate	1, 8, S, S, S, S ——— 7, 8, S, S	9, S, S, S, S, S – 4, 5, 5, 7, S	10, S, S, S, S, S — 7, 7, 14, S, S	1, 1, 2, 2, S, S ——— 7, S	1, 1, 1, 1, 1, 1
Sod. stearate	9, 9, 9, 13, S, S ——— 8, 9	8, 9, 9, 14, S, S ——— 9, 10	9, S, S, S, S, S – 8, 9, 9, S, S	8, S, S, S, S, S – 7, 8, 8, S, S	16, S, S, S, S, S — 7, 15, 19, S, S
Solgon	2, 6, 7, 8, S, S ——— 6, 7	S, S, S, S, S, S 6, 6, 6, 6, 6, 6	1, S, S, S, S, S – 3, 6, 6, 6, 6	1, 1, 1, S, S, S ——— 5, 6, 6	1, S, S, S, S, S – 3, 6, 6, 6, 6
Stearic acid	7, 7, 7, 8, 7, 9	4, 4, 6, 6, 8, 9	5, 6, 6, 6, 7, 7	4, 4, 5, 7, 9, 9	6, 6, 7, 7, 7, 9
Tetralene	1, 6, 6, 6, 6, 6	1, 6, 6, 6, 6, 6	6, 6, 6, 6, 6, 6	6, 7, 7, 7, 7, 7	9, S, S, S, S, S – S, S, S, S, S
Trimetso	2, 9, S, S, S, S ——— 6, 6, 7, 7	S, S, S, S, S, S 4, 4, 5, 5, 6, 7	3, 3, S, S, S, S ——— 6, 6, 7, 7	1, 1, 1, 2, 2, 3	1, 1, 1, 1, 1, 1
Undecylenic acid	10, 22, S, S, S, S ——— 7, 8, S, S	13, S, S, S; S, S — 7, 9, 9, 9, S	1, 3, S, S, S, S ——— 6, 6, 7, S	1, 1, 1, 2, 2, 3	1, 1, 1, 1, 1, 1
Zephiran	6, 6, 6, 6, 6, 6	1, 3, 6, 6, 6, 6	1, 1, 6, 6, 6, 6	1, 6, 6, 6, 6, 6	1, 1, 1, 1, 1, 1

Six mice inoculated i.p. with each dilution.

S = survival of a mouse.

The figures represent the number of days from inoculation to death.

In the second line the results are recorded of the immunity towards active virus.

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ORDINARY MEETING
of the Society held at
Manson House, 26, Portland Place, London, W.,
on
Wednesday, 4th November, 1942, at 4 p.m.

THE PRESIDENT
Sir RICKARD CHRISTOPHERS, *C.I.E.*, F.R.S., COL. I.M.S. (ret.),
in the Chair.

PAPER.

DYSENTERY IN THE MIDDLE EAST WITH SPECIAL REFERENCE TO SULPHAGUANIDINE TREATMENT

BY
COLONEL N. HAMILTON FAIRLEY, *C.B.E.*, F.R.S., A.A.M.C.
AND
COLONEL J. S. K. BOYD, *O.B.E.*, A.M.S.

CONTENTS

(3) The dysentery carrier and his detection.
(4) The bacteriological types and strains of *B. dysenteriae* implicated.
(5) Specific treatment.

II.—The Prevention of Dysentery.

From a military viewpoint it is more important to prevent than to cure dysentery, and for this reason brief reference to certain procedures which proved useful in the control and prevention of dysentery in forward military areas and fixed camps will be made. In some tropical countries, such as Java,* bacillary dysentery is predominantly a water-borne disease and prevention consists essentially in water sterilization. In the Middle East, however, there has been no difficulty in maintaining a safe water-supply, and infection is mainly fly-borne. In the desert, water exists only in the oases, but "faecal-feeding flies" abound everywhere and infection occurs mainly by their fouling food and drink. Uncooked vegetables, particularly lettuce, are always suspect, as human excrement is so often employed as manure.

It is proposed to comment here only on certain procedures which have proved useful in the control of dysentery in forward military areas and fixed camps in the Middle East.

(A) Forward Areas.

When troops take up new positions and are under fire or being bombed it is not always feasible to dig trench latrines immediately. In these circumstances troops are instructed to scoop out a shallow hole and after defaecation to cover the excreta immediately and completely with a layer of loose earth or sand not less than 2 inches in thickness. This prevents immediate access of flies but allows subsequent desiccation by the heat of the sun. It is the duty of the sanitary personnel to burn (if wood or fuel are available) or to treat promptly with disinfectants (which are also insecticides) and to bury all exposed human faeces and animal carcases found in the proximity of occupied areas. As soon as possible trench latrines are dug and, if available, fly-proof wooden covers are fitted; failing this, the instructions are that fresh excreta will be immediately covered with earth and all open latrines sprinkled with disinfectant or larvicide once daily. Borax has proved particularly effective owing to its diffusible properties, but unfortunately it was not always available. Once flies have laid their eggs in faeces, burial alone is insufficient to prevent breeding, as the young fly, after emerging from the puparium, can travel many feet through loose earth and sand; it is essential to make use of a larvicide or an insecticide.

An interesting observation made by Major Macnerras is that desert flies do not like entering any roofed structure which is darker inside than out. A

* In Java there is a big rainfall, much agricultural land is frequently flooded and the level of sub-soil water is high. The native Javanese defaecate directly into flowing water or streams running into rivers.

lean-to made of a tarpaulin of ground sheets proved useful in reducing the number of desert flies in field kitchens. Food must be protected from flies *en route* along lines of communication; failure to do this leads sooner or later to epidemic diarrhoea or dysentery. Finally, the immediate evacuation from forward positions where sanitation cannot be properly controlled of all persons suffering from "febrile" diarrhoea or diarrhoea associated with mucus or blood in the stools is a measure of the utmost value. When fighting is in abeyance, such troops may be treated in special dysentery wards in an advanced C.C.S.

(B) FIXED CAMPS.

In barracks and base hospitals where sewage and fly proofing are installed the control of dysentery should be a simple affair. In camps and hutted and tented hospitals many difficulties arise. The kitchen should be fly proofed: fish net curtains should be placed over the doors and windows if wire screening is not available. The regular use of fly swats, tangle-foot paper, formalin and sugar baits or sodium arsenite traps all proved valuable. While theoretically the detection and exclusion of "carriers" among cooks and other food handlers appears a promising method of preventing infection, in practice it is not of much use, partly because the work involved in carrying it out thoroughly is enormous, and partly because the man who is normal when he is examined may be an active case a few days later. It is more important to encourage such personnel to report sick should symptoms of any kind become apparent. They should be instructed to wash their hands regularly in some antiseptic solution such as lysol (1 in 40)—especially after defaecation. Frequent inspection of kitchens and latrines by combatant and medical personnel is helpful in keeping up a high standard of camp sanitation. Food coming from the kitchens should be protected from flies and net covers provided on the mess tables to protect milk, jam, sugar and the like. Where possible passages connecting the kitchen and mess should also be fly-proofed.

Fly-proof latrines with automatically closing lids are essential. If there is a pan system in operation supervision is necessary to see that the camp grounds are not fouled by the contents of the night-soil carts, and that the excreta are properly disposed of. The dumping of human excreta and garbage in the vicinity of camps may lead to a great increase in the fly population and to local outbreaks of dysentery or enterica.

Native labour employed near camps, especially when hutments were being built, proved a special menace. In order to prevent promiscuous defaecation, deep trench latrines of Moslem type were provided with fly-proofed superstructure and covers which automatically closed. These were sited so as to be readily available in different parts of the camp and full contract penalties were liable to be enforced for breaches of sanitary discipline.

(C) DYSENTERY WARDS IN HOSPITALS.

As far as possible in general hospitals dysentery cases were put in special dysentery wards. When feasible such wards were sited close to the laboratory, and their construction was modified with the object of minimizing cross infection and spreading the disease to other wards.

A very satisfactory plan was that devised by BRITTEN-JONES and described by HONE, KEOGH and ANDREW (1942). A wire-screened room in the rear of the ward contained a wire-screened cupboard to hold bed-pans, an incinerator for destruction of faeces and two copper boilers for sterilizing bed-pans. Incineration of the stools mixed with sawdust gave no objectionable odour. The latrines for ambulatory patients had their back opening into the incinerator room so that pans could be emptied directly into the incinerator. In wards of this type only one cross infection occurred in 600 cases; this was a patient with amoebic dysentery who contracted a Flexner V type infection while in hospital.

The medical officer in charge of the dysentery ward should gain experience in this type of work and should as far as possible be left for long periods in charge. He should be familiar with sigmoidoscopic technique and should work in close liaison with the pathologist, as the secret of success in diagnosis often lies in close co-operation between these two officers. Where cases are being treated in an advanced C.C.S., special dysentery wards are provided and it is a great advantage if the services of a mobile laboratory are made available; this measure, designed to ensure early treatment and the earliest possible return of cured cases to their units, worked well in quiescent periods when the demand for surgical beds was minimal.

III.—BACTERIOLOGICAL TYPES OF BACILLARY DYSENTERY ENCOUNTERED IN THE MIDDLE EAST.

The technique adopted by all laboratories in the Middle East for the isolation and identification of dysentery bacilli is the one which has been standard in the Army for a number of years. Organisms are classified into the non-mannitol and mannitol fermenting groups, the former containing Shiga's and Schmitz bacilli, the latter Sonne's bacillus and the Flexner-Boyd group. Antisera are provided which permit of the identification of the Shiga, Schmitz, and Sonne types, and two polyvalent sera which cover the Flexner group and Boyd I. A cross-section of isolations of the Flexner-Boyd group has been typed according to the classification described by BOYD (1940), but this procedure has not been carried out as a routine.

An analysis showing the percentage incidence in 8,665 cases of dysentery in which a causal organism was isolated is shown in the table on p. 258. These are the total isolations in 1941 in the laboratories of the Middle East Force.

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was common, while Strains I, III, IV and V were much rarer. It will be remembered that Flexner II corresponds to Andrewes' W, while Flexner VI is the Newcastle-Manchester group, variants of which show diverse biochemical reactions though possessing an identical antigen.

Certain members of the Boyd group are relatively common. Boyd I is picked out by the polyvalent serum and included with the Flexner series. A number of strains from the " other mannitol fermenters " have been collected and are being analyzed. Of these, a goodly number are late lactose or saccharose fermenters, a group which may be regarded as non-pathogenic. Appreciable numbers are Boyd II. Another strain well known in India as P274, but *sub judice* as to pathogenicity, also forms a considerable portion of this series.

B. dysenteriae Shiga proved, next to the Flexner group, the most frequent cause of dysentery; the incidence varied in different areas, the average being just over 15 per cent.

ANNUAL PERCENTAGE OF ISOLATIONS—1941.

Total Isolations	8,665	
E. histolytica	12·3	per cent.
B. dysenteriae Shiga	15·8	,,
Schmitz	5·2	,,
Sonne	6·3	,,
Flexner-Boyd I. ...	52·3	,,
Other non-mannitol fermenters ...	3·6	,,
Other mannitol fermenters	4·5	,,

Infections with *B. dysenteriae* Schmitz and *B. dysenteriae* Sonne were less common.

Other strains having the biochemical reactions of *B. dysenteriae* Shiga and Schmitz, but of different antigenic structure, *i.e.*, not agglutinated by Shiga or Schmitz antiserum, were not infrequently isolated from dysenteric stools. An investigation of these strains is now nearing completion.

That at least twelve different organisms may be responsible for an attack of bacillary dysentery is rarely appreciated by clinicians. This is illustrated by the fact that in selecting comparable series of cases to control the effects of specific drug treatment it is common to find as few as thirty considered adequate. Naturally, where there are twelve variable aetiological agents a very large series of cases is necessary in both groups if cases are selected on a clinical and not a bacteriological basis.

IV.—IMPORTANT ASPECTS OF LABORATORY DIAGNOSIS.

(A) SELECTION OF SPECIMENS.

No amount of technical skill on the part of the pathologist can make up for defective material supplied from the wards.

For the successful isolation of dysentery bacilli specimens must be obtained absolutely fresh and preferably as early as possible in the course of the disease. The highest percentage of isolations is made from cases which have not passed the stage of watery diarrhoea, in which flakes of cellular mucus are to be found. The chances of successful isolation are reduced once brown faecal matter reappears in the stools, and with the older types of medium (McConkey, litmus-lactose agar, etc.) it is a waste of time and material to culture formed or semi-formed stools in which there is no visible mucus. More successful results are now being obtained with the new desoxycholate medium, but adequate supplies are not yet available for it to be brought into general use. If the specimen is sent to the laboratory in a bed-pan the latter must be free from antiseptic and not contain urine which should be passed separately. Most of these facts were well established during the last war, yet failure to isolate *B. dysenteriae* in the present campaign was repeatedly traceable to neglect of these elementary principles.

Where laboratory facilities are not available on the spot and the specimen has to be sent some distance, a small portion of mucus collected from a fresh stool should be placed in a screw-capped bottle containing a 30 per cent. solution of glycerine in normal saline adjusted with sodium phosphate to pH 8, and tinted with phenol red ; this indicator gives warning of subsequent development of acidity in the glycerine which itself prevents growth of *B. dysenteriae*. Provided the right material has been selected, the use of this preservative will enable cultures to be obtained some 12 to 18 hours after passage of the stool.

(B) MICROSCOPIC APPEARANCES.

The most striking feature in the microscopic examination in the early stages of bacillary dysentery is the extreme cellularity of the mucus. Instead of being more or less structureless and homogeneous, as its macroscopic appearance suggests, it is found to consist of a mass of closely packed cells. Red blood cells vary according to the portion examined ; if a blood-flecked piece be selected they will naturally be very numerous. Exclusive of erythrocytes, 90 per cent. or more of the remaining cells are pus cells, *i.e.*, polymorphonuclears ; they are usually in a good state of preservation, but in old specimens may show some pyknosis. The remaining 10 per cent. of cells is made up chiefly of shed epithelial cells, mononuclears and macrophages and a few eosinophils. The macrophages, presumably endothelial in origin, are large mononuclear cells which may contain ingested cell debris, fat globules and even red cells. They may show feeble movement, but never the active motility characteristic of *E. histolytica*. Muco-pus with these microscopic characters constitutes the so-called " bacillary exudate " and is essentially an acute inflammatory exudate. As the case advances the cellular composition of the exudate changes. The number of cells decreases, the pus cells become degenerate and proportionately

less numerous, red blood cells become scanty or absent, and mononuclear cells increase. This is known as " indefinite exudate " and is not unlike the exudate encountered in amoebic dysentery. It has no diagnostic significance.

V.—Sigmoidoscopy.

The sigmoidoscopic appearances during the acute stage of bacillary dysentery have been well described by Biggam and Arafa (1930) and Manson-Bahr (1939) but little information is available concerning the appearance of the bowel in cases in which resolution is incomplete. Healing of the lesions of acute bacillary dysentery begins when the infection is overcome and acute symptoms subside, and in most cases is complete in periods ranging up to a fortnight. Occasionally this process is delayed, and in the present investigation the condition of the bowel in these cases was specially studied. The appearances in acute cases which fail to clear up in three weeks, and in chronic cases where symptoms have been present for a period of months, will be described.

From the diagnostic viewpoint not only do the appearances of the bowel wall frequently suggest the nature of the infection, but by swabbing the ulcers ideal material can be obtained for culture and determining the type of exudate, while gentle curettage of the ulcer surface will supply material enabling vegetative forms of *E. histolytica* to be demonstrated if amoebiasis be present.

(a) Acute Dysentery.

In the early stages in mild infections the mucosa is intensely red and hyperaemic and exudes an excess of mucus; these changes may be most marked in the rectum. In moderately severe cases the mucosa is more intensely inflamed and bleeding occurs on instrumentation; the bowel wall is infiltrated and oedematous and there is some degree of rigidity and inelasticity. Blood-stained mucus or muco-pus is present. In still more severe cases localized patches of greyish membrane may be visible consisting of necrosed mucosa infiltrated with fibrin. These, on separation, leave a raw, granulating, bleeding, ulcerated surface. In fulminating cases (usually Shiga infections) there is extensive greyish or greyish-green membrane formation, due to coagulation necrosis, associated with areas of haemorrhage and intense inflammation.

Sigmoidoscopy during the acute stage of this disease may be painful and dangerous, and as the information obtained is of academic interest rather than of practical value, it should not be undertaken during the first 14 days of the attack unless special indications exist.

(b) Subacute or Slowly Resolving Dysentery; and Carriers.

In cases where dysenteric symptoms have persisted longer than the third week, a variety of lesions was observed, some of which have not previously been described.

(1) The mucous membrane may remain universally inflamed, bleeding on instrumentation, while scattered here and there over the mucosa are irregular superficial ulcers covered with muco-pus.

(2) The mucosa may be normal, or only limited areas may show persisting inflammation, but ulcers presenting a variable appearance remain. The purulent exudate from such ulcers is bacillary in type, and culture frequently reveals *B. dysenteriae*—especially of the Flexner group—despite the fact that former bacteriological examination of the stools may have been negative. The pus may be obtained by direct swabbing of the ulcers or it may ooze up from the inflamed, pitted and ulcerated mucosa from pressure exerted directly on the bowel wall by the sigmoidoscope. Apart from red granular patches showing superficial ulceration or pitting, the following lesions may be found :—

(i) Small discrete nodular or papule-like lesions with yellow crusts surrounded by a zone of oedematous hyperaemic mucosa. On scraping off the yellow crusts a bleeding crater is left containing exudate of bacillary type from which *B. dysenteriae* may be cultured.

(ii) Numerous punched-out pock-like ulcers bathed in purulent exudate which involve the mucosa and may show no generalized inflammation. The ulcers are close set and, on swabbing away the pus, their clean-cut vertical walls are very striking.

(iii) Small superficial clean-cut ulcers with well-defined edges, or apthae-like ulcers with yellowish-white exudate which are situated chiefly on the mucosal folds or valves of Houston. The bowel wall distends readily, there is no inflammation of the intervening mucosa and the lesions are liable to be mistaken for those found in amoebic dysentery. Scrapings from the ulcer base, however, do not contain *E. histolytica*, and culture reveals *B. dysenteriae*.

Patients with lesions of this type may complain of occasional attacks of looseness of the bowels for weeks or even months following their original attack of dysentery, but culture of the stools frequently yields negative findings. Sigmoidoscopy is the best means of recognizing such " carriers " and if this is not done they are liable to be discharged to their unit and disseminate infection. In our opinion (1) all bacillary dysentery carriers have lesions of this or a similar type somewhere in the colonic mucosa, (2) the bacilli are derived from the ulcers and (3) once the ulcers have completely healed, dysentery bacilli disappear from the stools.

(c) CHRONIC DYSENTERY.

ROGERS (1921) found that with few exceptions the lesions of chronic bacillary dysentery were limited to the lower half or two-thirds of the large intestine. Two types were encountered :—

(1) In severe chronic bacillary cases lasting 3 months or longer, the large bowel may be tubular in outline, the walls thickened and contracted and difficult

to distend. The surface is composed of red granulation tissue which bleeds readily on instrumentation. Sometimes a polypus-like condition is produced by the heaping up of exuberant granulation tissue. The general appearances closely resemble those found in chronic ulcerative colitis, and in advanced cases the patulous condition of the anus, the atrophic appearance of the perianal skin and the wasting of the gluteal and perineal muscles are common to both diseases.

(2) In other cases of chronic dysentery in which the bowels had acted five or six times daily for 6 months or longer, superficial oval or circular ulcers up to 1 cm. in diameter were found scattered over an apparently normal mucous membrane. *B. dysenteriae* Shiga was isolated from cultures of material obtained by swabbing the ulcer surface.

(D) HEALING STAGES OF DYSENTERY.

In the healing stages and during convalescence the mucous membrane may be red or rose-pink, oedematous and slightly nodular in appearance; when epithelialization is proceeding those areas of mucosa which are not yet covered by their normal thickness of epithelium appear scarlet-red and somewhat dry, while in other areas milky or branny patches and leucoplakia-like appearances may be found, due to irregular and excessive local epithelial proliferation. Widespread pitting at the site of former ulcers is very characteristic, and it is often possible on this evidence to state definitely that the condition was one of bacillary dysentery.

(E) SUMMARY.

Sigmoidoscopy reveals the pathological changes in the colon during life. In our opinion it plays a valuable role in the diagnosis of the dysenteries, and material collected for laboratory examination during sigmoidoscopy may yield positive findings where previous investigations of the faeces have proved negative. In bacillary dysentery sigmoidoscopy has an additional value as it may reveal the persistence of ulceration and a carrier state at a time when the clinical features and solitary stool examination suggest that cure has resulted. When practicable every dysentery patient should have a sigmoidoscopical examination made before discharge from hospital. Repeated consecutive naked-eye examinations of the whole stool, however, provided they are carefully carried out, will sooner or later reveal the presence of mucus or muco-pus.

VI.—Clinical Aspects of Dysentery.

(A) DYSENTERY OF FLEXNER TYPE.

Much of the Flexner dysentery seen in the Middle East is mild in type and as a rule symptoms of severe toxaemia and dehydration do not develop; the same holds to a lesser degree with Shiga infections. The onset is characterized by colicky abdominal pain followed by urgent diarrhoea. Nausea, transient

vomiting, headache, shivery feelings and aching in the limbs may accompany, follow or precede the onset of these abdominal symptoms, but, as recently stressed by HONE, KEOGH and ANDREW (1942), the presence of mild prodromal toxic features preceding the onset of diarrhoea affords no index to the severity of the infection.

Fever is present in most cases on admission to hospital. Examination reveals diffuse tenderness, most marked in the right or left iliac fossa or the upper part of the abdomen. The abdominal pain is predominantly colicky in nature; between attacks the patient may get relief or complain of a persistent dull ache. Tenesmus due to rectal spasm is less frequent in mild than in severer infections.

Diarrhoea is generally most intense on the first and second day of attack, the stools as a rule decreasing in number thereafter, but in some cases the maximum number is reached on the third, fourth or fifth day. At first the stools are fairly copious and watery, but if looked for, flakes of mucus and blood will generally be found. Most cases of " febrile " diarrhoea in which numerous liquid stools are passed are bacillary dysentery. As the condition develops the stools are found to consist of mucus or of blood and mucus only. The mucus is glassy like egg-white, practically odourless, tenacious and sticks to the bed-pan. The blood varies in quantity and may be seen as mere flecks of blood in otherwise colourless mucus or may be more copious, when it may tinge the mucus a reddish colour. In more severe cases the mucus becomes definitely purulent in character. As the case advances towards recovery blood disappears, mucus becomes scantier and thicker and brown faecal matter reappears. Fever and diarrhoea may last only 1 or 2 days in mild cases, in others it goes on for a week or more. Should pyrexia or diarrhoea with mucus persist more than a fortnight, sigmoidoscopic examination should be made. The findings in subacute and chronic bacillary dysentery are outlined later.

(B) TYPES OF SHIGA DYSENTERY ENCOUNTERED.

(1) Acute fulminating cases of Shiga dysentery as described in textbooks were rare, but death in one case occurred within a period of 32 hours. The onset in this case was sudden with vomiting and severe diarrhoea, frequent fluid brown stools being passed. The patient rapidly became collapsed and dehydrated, muscular cramps ensued and death supervened before the frequent small muco-sanguineous stools characteristic of bacillary dysentery developed. Gastro-enteritis and later food poisoning were diagnosed, but culture of the stools revealed *B. dysenteriae* Shiga, and at autopsy extensive coagulation necrosis of the colonic mucosa was found.

(2) In severe Shiga infections the onset may be sudden or insidious. In the former case the illness commences with a rigor or feeling of chilliness associated with fever, headache, nausea or vomiting, colicky abdominal pain

and frequent urgent bowel actions. Tenesmus commonly follows. At first the stools are fluid in consistency, brown in colour and contain only flecks of mucus or blood, but soon they became mucoid or mucopurulent in character, often contain blood and occasionally greenish sloughs of mucous membrane. The presence of considerable quantities of blood or of greenish sloughs in the stool should always suggest a Shiga infection. As the condition progresses the bowels may act twenty to sixty times daily.

In cases with an insidious onset severe intestinal symptoms and fever may not supervene for several days. As the infection progresses and more mucosa is destroyed, toxaemia increases. The cheeks then become flushed, the eyes bright, the expression anxious, fever increases, the pulse is more rapid and the tongue coated and yellow. Restlessness, sleeplessness and delirium develop. When fluid loss continues without compensation, dehydration results; there is then increasing thirst, a dry brown tongue, muscular cramps, a dry shrivelled skin, collapsed peripheral veins, a feeble rapid pulse, a low blood pressure, oliguria and an increase in nervous symptoms. Occasionally renal failure complicates the picture. There is albuminuria, granular casts and nitrogenous retention. Oliguria, abdominal distention and hiccough are characteristic. In the most severe cases anuria supervenes and the patient dies in uraemic coma. Another complication is peripheral circulatory failure associated with decreased blood volume which arises from a combination of toxaemia, dehydration and possibly hypoprotinaemia consequent on the loss of serum protein through the extensively ulcerated colonic mucosa; it is generally fatal. Sometimes infection of the colon extends more deeply involving the muscular coat and even the peritoneum which becomes inflamed; chronic peritonitis with serous effusion results. Under these circumstances the abdomen becomes distended, the abdominal muscles tender and somewhat rigid, and there may be dullness in the flanks or free fluid demonstrable. Flatulence, vomiting and colicky abdominal pain often prove troublesome. A polymorphonuclear leucocytosis is characteristic. The condition is non-surgical and must be differentiated from acute peritonitis, secondary to perforation, which demands immediate laparotomy.

(3) The less severe infections present much the same picture as dysentery of Flexner type already described and can only be diagnosed by bacteriological methods. Clinically they cannot be differentiated.

Course.—One unfortunate feature of Shiga dysentery is that neither the mode of onset, nor the subsequent febrile course necessarily affords any reliable index to prognosis. The rare fulminating type with collapse and subnormal temperature rapidly dies, and the toxaemic patient with sustained high fever of intermittent or remittent type is obviously very ill. Other types, however, occur which unexpectedly end fatally. Thus patients may develop first diarrhoea, then mild dysentery, and remain afebrile for several days; suddenly fever and severe dysenteric features develop and death ensues 7 to 14 days from the onset of what appeared originally to be a mild infection. In others there

may be an initial period of fever lasting 10 to 14 days, after which the patient becomes afebrile, dysenteric symptoms continue, the condition slowly deteriorates and the patient dies some 4 to 8 weeks after onset. At this last stage the stools may consist of brownish fluid with relatively little mucus, yet from this mucus *B. dysenteriae* Shiga can be isolated. In such cases the destruction of the mucosa is so great at its terminal stage that both water absorption and mucus secretion are seriously interfered with, and the contents of the small bowel are little changed in their passage through the colon.

(C) SUBACUTE AND CHRONIC BACILLARY DYSENTERY.

In the subacute and chronic stages of bacillary dysentery looseness of the bowels and the passage of stools showing traces of blood and mucus persists. Three groups need differentiation :—

(1) *Those with superficial ulcers scattered throughout a more or less normal looking mucous membrane or one which is subject to patchy inflammation only.* Culture from swabs of the ulcers usually reveals the causative organism. The various types of ulcers which may be encountered have already been described in the section on sigmoidoscopy and need no further comment here.

(2) *Those resembling chronic ulcerative colitis and presenting generalized mucosal inflammation and deep involvement of the bowel wall.* Here there is an extensively ulcerated raw red granular bleeding surface with a rigid non-dilatable bowel which has lost its normal folds. X-ray shows a straight tubular type of colon with loss of haustration. Cultures taken from the bleeding mucosal surface may reveal the causative organism.

(3) *Those in which there is no actual ulceration, but patchy redness of the mucosa and spasm may be noted.* Swabbing of the hyperaemic red areas may lead to erosion and mild bleeding. Cultures fail to reveal *B. dysenteriae*, there is no typical " bacillary exudate," mucus secreted is not markedly cellular and the persisting looseness of the bowels is mainly attributable to an irritable colon which is the aftermath of the original infection. This condition is not ameliorated by sulphaguanidine therapy whereas types (1) and (2) are. A similar condition is occasionally encountered in acute cases treated with sulphaguanidine, and here the red hyperaemic patches seen on sigmoidoscopy are due to incomplete epithelialization over former ulcerated areas. In other treated cases, though healing of the mucosa is complete, structural damage in the outer coats of the bowel (fibrosis) persists with resulting colonic dysfunction. Conditions due to (a) persisting bacillary infection, (b) defective epithelialization or (c) actual fibrosis in the deeper layers of the bowel wall must be differentiated from post-dysenteric diarrhoea of psychogenic origin.

After an attack of dysentery neurotic patients are liable to become bowel-conscious and introspective and may continue to pass several loose or fluid stools each day for many months. Their mentality is characteristic of the neurotic, their nutrition generally satisfactory, sigmoidoscopy and radiological

investigations reveal no abnormality; blood, cellular mucus and muco-pus are absent from the stools and culture of the faeces fails to reveal any pathogen.

VII.—Complications.

Complications in both Shiga and Flexner types of bacillary dysentery were infrequent and were mainly encountered in the severer infections. Certain of these seen in the Middle East have not previously been recorded in bacillary dysentery and for convenience they may be classified as follows :—

(A) INTESTINAL AND ABDOMINAL.

(1) Intestinal haemorrhage.
(2) Intestinal perforation with peritonitis.
(3) Chronic peritonitis with localized or generalized effusion of peritoneal fluid.
(4) Pneumoperitoneum (one case).
(5) Portal pyaemia and multiple abscesses of liver (one case).
(6) Haemorrhoids which may become thrombosed.
(7) Rectal prolapse.

(B) SYSTEMIC.

(1) Peripheral circulatory failure.
(2) Renal failure.
(3) Toxic arthritis.
(4) Peripheral neuritis.
(5) Conjunctivitis and iritis.
(6) Pneumonia.
(7) Parotitis.
(8) Petechial and purpuric rash.

(A) INTESTINAL AND ABDOMINAL COMPLICATIONS.

Intestinal Haemorrhage.—A certain amount of blood in the stools is characteristic of bacillary dysentery. In some instances, however, the quantity of fresh blood is considerable and may be so excessive as to produce rapid anaemia and other clinical manifestations of acute blood loss. Severe haemorrhage is always suggestive of Shiga infection. Such cases should be treated with sulphaguanidine, morphia and continuous drip blood transfusion. On several occasions the haemorrhage was so severe that caecostomy was performed with the idea of resting the bowel and stopping the bleeding. Surgical intervention of this type is undesirable as caecostomy does not rest the colon ; it is also unnecessary as medical treatment suffices.

Intestinal Perforation.—In the majority of cases of bacillary dysentery the inflammation is superficial and confined to the mucosal layer. Perforation, therefore, is rare, but it can and does occasionally occur as a terminal complication in seriously ill patients with the production of acute peritonitis. It is specially liable to occur in cases in which the dysenteric infection is superadded to a pre-existing deficiency disease such as pellagra. There is always extensive

ulceration throughout the colon. Surgical intervention is the only possible means of dealing with such a condition, but the prognosis is invariably grave. No such case in our series recovered.

Chronic peritonitis with localized and generalized effusion of sterile peritoneal fluid occasionally develops in the later stages of bacillary dysentery when infection has extended deeply into the bowel wall and involved the muscular coats. The clinical features have already been considered.

Pneumoperitoneum developed during the course of one case of bacillary dysentery contracted in Palestine. The mechanism of its production was not determined. Liver dullness was completely obliterated in the recumbent posture. Radiological investigation clinched the diagnosis. In another case portal pyaemia with multiple liver abscesses was found at autopsy associated with colonic ulceration due to *B. dysenteriae*. Secondary bacterial infection was probably responsible.

The straining at stool is not infrequently associated with haemorrhoids which occasionally became thrombosed. Rectal prolapse was also recorded.

(B) SYSTEMIC COMPLICATIONS.

Peripheral circulatory failure has already been referred to (p. 264).

Renal failure, which has clinical resemblance to that found in traumatic anuria (crush injuries), results from a combination of factors in which dehydration and toxaemia play an important part, leading as they do to reduced glomerular filtration and degenerative changes in the tubules. The urine contains granular casts and albumin, and there is azotaemia and oliguria which may pass on to anuria; finally, uraemia may develop. Oedema and haematuria were not observed. Renal failure of this type occurs in patients who have never received sulphaguanidine and therefore cannot be attributed to this drug.

The histological changes which occur in the kidneys have been studied in fatal cases by Major J. C. Dick (1942) who has found, in a proportion of these, glomerulo-nephritis of varying degrees of severity.

Toxic arthritis, which was observed in both Flexner and Shiga dysentery, was far from common. It took the form of effusions into the joints and periarthritis and generally came on in the third, fourth or fifth week or during convalescence. The large joints such as knees, ankles, elbows, shoulders and wrists were prone to be attacked. It sometimes persisted for many weeks and its course did not appear to have been favourably influenced by sulphaguanidine treatment once the arthritis had developed. No permanent deformity was noted and recovery was ultimately complete.

Peripheral neuritis was rare. In such cases vitamin B_1 therapy is indicated; dietetic restrictions employed for therapeutic purposes may predispose.

Conjunctivitis was seen in a number of cases. Like arthritis it generally came on later in the course of the disease or during convalescence, was bilateral and mild in type and generally cleared up quite satisfactorily in about a week.

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Pneumonia was not an infrequent complication in fatal cases.

Parotitis was recorded, but was uncommon in hospital-treated patients.

Petechial and purpuric rashes were only encountered in severely debilitated or gravely ill patients.

VIII.—THERAPY.

The majority of cases of bacillary dysentery of all types in the Middle East recovered within reasonable time with rest in bed, copious fluids, appropriate dietary and limited sodium or magnesium sulphate therapy. A minority—especially cases of Shiga dysentery—failed to do so: some died, others passed into a state of subacute and chronic dysentery, while others became carriers.

(A) SODIUM OR MAGNESIUM SULPHATE.

As a general rule in routine treatment the initial dose was 2 drachms. Subsequently 1 drachm was given 2-hourly for the first 12 to 24 hours and then 4-hourly, 6-hourly and finally 8-hourly for the next 3 or 4 days or until the stools became faeculent. As a rule it proved inadvisable to continue sulphate therapy longer than 5 days as in many cases it merely kept up the diarrhoea.

(B) DIETARY.

For the first 24 to 36 hours only water should be allowed to which may be added glucose, lactose or cane sugar. Subsequently albumin and barley water, tea and chicken broth are given followed by soups, marmite, orange juice, jelly, dry biscuits, toast, clear honey or golden syrup, whey and apple purée. This is followed by arrowroot, cornflour, ground rice, sago pudding and as improvement continues by eggs, non-fatty fish, chicken, butter, milk drinks, junket and stewed fruit. This type of low residue diet is advisable in severe cases—especially Shiga infections—and in patients who are having sulphaguanidine therapy. HONE, KEOGH and ANDREW (1942), who were dealing mainly with mild dysentery of non-Shiga type in Egypt, found that milk drinks introduced at an early stage in no way prejudiced the patient's recovery. Two types of diet were tested. In one a diet of clear fluids and jelly was used until the stools were reduced to six to ten per day, at which stage toast and cereals were allowed. When the patient was having three or four daily motions protein was added to the diet as well as fats in small quantities. A week after admission to the wards most patients were receiving the ordinary hospital light diet.

The other diet differed in so far as milk drinks were the first addition to the initial diet of clear fluids and jelly, after which milk foods were allowed as well as cereals. There was no evidence of fat intolerance in either group of patients and both did equally well.

(c) ANTI-DYSENTERIC SERUM.

Experience was limited to three types of anti-dysenteric serum :—

(1) Multivalent anti-dysenteric serum (Lister Institute serum). 1 c.c. = 2,000 I.U. Shiga anti-toxin.

(2) Polyvalent anti-dysenteric serum (Commonwealth Serum Laboratory).

(3) Refined anti-dysenteric Shiga serum (B.W. & Co.). 1 c.c. = 20,000 I.U. Shiga anti-toxin.

(1) *Polyvalent Anti-Dysenteric Serum.*

O'Brien (1940) in his review of the treatment and prophylaxis of bacillary dysentery concluded that "if satisfactory anti-sera to the Flexner group of bacilli can be produced, there are as yet no clinical or laboratory figures to convince us that sera at present available are of value." Experience gained in the Middle East produced no satisfactory evidence that either the multivalent or polyvalent serum was of therapeutic value. Serum sickness, however, was common occurring in at least 20 per cent. of patients receiving such treatment. Its chief manifestation was a troublesome urticarial eruption which was not infrequently associated with joint pains and fever, while occasionally actual effusion into the joints occurred. Serum sickness caused considerable inconvenience ; it often came on just as the patient was recovering from his bout of dysentery and prolonged the stay in hospital. On the other hand, true anaphylactic shock manifesting itself immediately after the injection was extremely rare.

In view of (1) the dubious value of polyvalent anti-dysenteric serum, (2) the untoward reactions which frequently follow its use, (3) the mild nature of most non-Shiga bacillary dysentery, (4) the recent introduction of specific drugs, *i.e.*, sulphaguanidine and allied sulphonamides, it is evident that the polyvalent anti-dysenteric sera are likely to play a decreasing role in the treatment of this disease. In the Australian Forces polyvalent serum therapy is no longer advised.

(2) *Refined Anti-Dysenteric Shiga Serum* (1 *c.c.* = 20,000 *I.U.*).

O'Brien (1940) pointed out that there was no reason why monovalent anti-dysenteric Shiga serum of 15,000 to 20,000 I.U. per c.c. should not be produced in limited quantity either by the ordinary ammonium sulphate concentration process or by the Pope process of partial peptonization. Subsequently an experimental batch was produced by the latter method containing 20,000 I.U. per c.c. and taken by one of us (N. H. F.) to the Middle East for investigation.

Its use was restricted to proved Shiga cases ; it was given intravenously in a dosage of 5 to 10 c.c. (*i.e.*, 100,000 to 200,000 I.U.) and repeated daily when thought advisable. No serum reactions were noted in a series of over thirty cases receiving such treatment.

At first we were favourably impressed. Patients undoubtedly felt better, stigmata of toxaemia decreased, abdominal colic was lessened and for a period

of 24 to 48 hours the clinical improvement was definitely evident. Unfortunately the benefit was too often transient and improvement was not maintained. This was illustrated early in the case of two medical officers who contracted fatal Shiga dysentery in Egypt.

Case I.—The onset was insidious with simple afebrile diarrhoea, about the 6th day fever developed, blood and mucus were found in the stools and *B. dysenteriae* Shiga isolated. The patient went into hospital where he was not considered to be sick enough to justify anti-dysenteric serum therapy.

By the 9th day he was very ill ; hiccough and intestinal distension were troublesome features and 100,000 I.U. of refined anti-dysenteric Shiga serum were administered intravenously. Temporary improvement followed. Next day another 100,000 units were given. Periphery circulatory failure developed on the 11th day and death rapidly followed.

At autopsy extensive dysenteric ulceration of the colon was found, the mucosa having been almost completely destroyed.

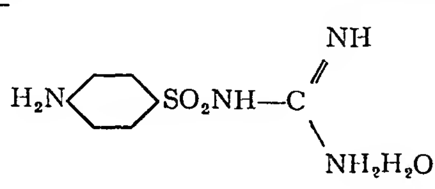
Case II.—The onset was sudden with rigor, generalized pains, high temperature, severe colicky abdominal pain and urgent diarrhoea followed by stools which soon consisted of blood and mucus. The patient was immediately put to bed, sulphate treatment started and multivalent anti-dysenteric serum (Lister Institute) given. Following the isolation of *B. dysenteriae* Shiga, numerous intravenous injections of refined anti-dysenteric Shiga serum were given—in all about 2,000,000 I.U. throughout the course of an illness which lasted over 2 months, and was complicated latterly by mild chronic peritonitis with a localized effusion into the peritoneal cavity. The clinical course remained quite unmodified by serum therapy and Shiga's bacillus was cultured from the stools at different times throughout the course of the disease.

The impression obtained regarding the therapeutic value of refined anti-Shiga serum was that it caused temporary clinical improvement by neutralizing Shiga exotoxin, but that it failed to modify the course of severe Shiga infections owing to lack of any bacteriostatic or bacteriocidal action. Sulphaguanidine exerts such action, and it is our opinion that refined anti-dysenteric Shiga serum should be reserved for (1) fulminating or severely toxic cases immediately on admission to hospital, (2) for Shiga cases where sulphaguanidine treatment alone has failed. The dosage should be not less than 200,000 I.U. given intravenously; the injection should be repeated in 12 hours in fulminating or severely toxic cases and sulphaguanidine therapy should be carried out simultaneously.

The action of sulphaguanidine and anti-dysenteric Shiga serum in Shiga infections are mutually complementary since sulphaguanidine exerts a bacteriostatic action on the dysentery bacilli in the gut wall and intestinal contents, while the Shiga anti-toxin neutralizes Shiga exotoxin in the tissues and circulating blood.

(D) SULPHAGUANIDINE TREATMENT

Sulphaguanidine (p-amino-N-guanylbenzene sulphonamide) is a guanidine analogue of sulphapyridine, sulphathiazole and sulphadiazine and has the following formula :—



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(1) *Pharmacological Properties.*

MARSHALL, BRATTON, WHITE and LITCHFIELD (1940) described its preparation, pharmacological properties and toxicity, also its bacteriostatic effect on various bacteria *in vivo* and *in vitro.* Though moderately water-soluble (200 mg. per 100 c.c.) it is insoluble in strong alkali and no sodium salt is formed. They found that a high concentration could be obtained in the intestine associated with a low concentration in the blood (2 to 5 mg. per 100 c.c.) and tissues, and suggested that it possessed properties which should make it valuable in the treatment of bacterial infections of the alimentary tract.

Absorption.—In mice, when sulphaguanidine was given *per os*, the blood concentration curves were nearly the same for small and for large doses, which was quite different from what had been found with the other sulphonamides like sulphanilamide, sulphapyridine, sulphathiazole and sulphadiazine.

In man, too, sulphaguanidine is absorbed to a large extent when very small doses are given, but to a small extent when larger doses are given. MARSHALL (1942) suggests the reason for this is that it is absorbed slowly and only in the small intestine.

Excretion.—The ratio of urinary excretion in the dog is three times as rapid as that of sulphanilamide, 95 per cent. of the drug given intravenously being excreted in 24 hours. Sulphaguanidine is conjugated to a variable degree; but fortunately both it and acetylsulphaguanidine are soluble in urine at a pH 7·1, but, unlike most other sulphonamides, they do not show any increase in solubility as the pH rises. For this reason there is no advantage in alkalinizing the urine with a view to prevent deposit in the tubules. A " tide of crystals " may appear in the urine during the first week, but not all these crystals are necessarily sulphaguanidine, heavy deposits of urates and phosphates sometimes contributing. Acetyl derivatives may under certain circumstances be deposited in the renal tubules, but fortunately the crystals are soft and the deposit does not form hard renal concretions as most other sulphonamide derivatives do. In our first series of 371 cases of bacillary dysentery treated with this drug there was no instance of renal complications such as macroscopic haematuria due to this cause.

Toxicity.—Sulphaguanidine is undoubtedly less toxic than the other sulphonamide drugs in common use. The toxic reactions observed in our series included headache, malaise, skin rashes and mild pyrexia. Haematuria, haemolytic anaemia, agranulocytosis and jaundice were never observed. Mild

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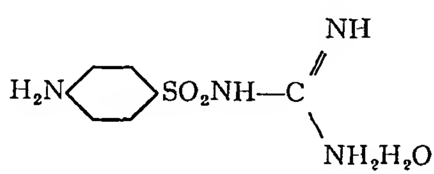

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deemed necessary when there is severe colicky abdominal pain, tenesmus or very frequent stools, *i.e.*, thirty or more in 24 hours. By slowing down the rate of peristalsis, opiates should tend to prolong the action of sulphaguanidine and so increase its local bacteriostatic effect. Heat in the form of hot-water bottles, antiphlogistine or turpentine stoups applied to the abdomen may be helpful for the first 24 hours before sulphaguanidine has exerted its maximal beneficial effect.

Strict dietary is enforced along the lines previously discussed. A low residue diet is naturally advisable and in severe Shiga infections milk drinks are not permitted until completion of the course of treatment. Dehydration must be prevented by giving as much fluid *per os* as can be comfortably taken. When indicated intravenous injections of saline (0·85 per cent.) and glucose (5 per cent.) solution are administered either intermittently or by continuous drip.

In the terminal phase where there is peripheral circulatory failure, or in the later stages in severely ill patients where copious fluid stools are being passed, liquid serum or reconstituted plasma may be of value.

Blood transfusion should be given in the acute stage where there is severe intestinal haemorrhage or later where severe anaemia has developed.

(4) *Indication for Sulphaguanidine Treatment.*

The first batch of sulphaguanidine available in the Middle East was obtained by Major BUTTLE from Dr. MARSHALL. Subsequently larger supplies were received, but the drug was never available in a quantity sufficient either to do a large-scale control experiment or to treat cases immediately dysenteric symptoms appeared, which is the therapeutic ideal from the army point of view. Were supplies unlimited, cases could be diagnosed on clinical grounds,* and, although specimens would still be sent to the laboratory for culture and microscopical reports whenever possible, the medical officer would not await this report before initiating treatment. Such early treatment should limit the amount of structural damage done to the colon, shorten the stay in hospital, largely eliminate the carrier and save life in a number of Shiga infections of severe type. Where supplies of sulphaguanidine are limited its use should be restricted to :—

(i) Cases of proved Shiga dysentery.

(ii) Dysentery of any bacillary type in which (*a*) the intestinal features are severe, especially if associated with much blood in the stools, or (*b*) where the stigmata of toxaemia or dehydration are marked.

(iii) Dysentery with severe onset as indicated by (*a*) collapse with

* Any case suddenly developing colicky abdominal pain, urgent diarrhoea with flakes of mucus or naked-eye mucus and blood in the stools and fever, should be regarded as bacillary dysentery until proved otherwise. A bacillary type of exudate, even in the absence of positive culture of *B. dysenteriae* is regarded as diagnostic if reported in such a case.

conjunctivitis was recorded in a few patients undergoing treatment, but as this is a recognized complication of bacillary dysentery there is no certainty of its causal relationship to sulphaguanidine administration.

(2) *Dosage.*

The dosage in acute cases is as follows :—

(1) An initial dose of 0·1 gramme per kg.

(2) A maintenance dose of 0·05 gramme per kg. 4-hourly for the period during which the number of stools passed exceeds five per day.

(3) A further maintenance dose of 0·05 gramme per kg. every 8 hours until the stools have become normal in number (one to two) and consistency for 2 days.

The duration of treatment should not exceed 14 days. If necessary the course can be repeated.

In most cases of *chronic* dysentery presenting isolated ulcers or a sigmoidoscopic picture resembling ulcerative colitis in which the bowels were acting less frequently (three to eight times daily) 0·05 gramme per kg. were given every 4 hours for 5 days, followed by 0 05 gramme per kg. subsequently every 8 hours for another 5 days. In others 0·05 gramme per kg. were given every 8 hours from commencement of treatment which lasted 5 to 7 days. This is the dosage advocated by MARSHALL, and in his opinion should suffice to keep the faeces saturated with sulphaguanidine without leading to excessive absorption and undue blood concentration. In our experience this dosage has not always proved adequate in chronic dysentery—especially where there is generalized inflammation of the colon with deepseated changes in the bowel wall. Here a dosage of 0·1 gramme per kg. every 8 hours for the first 5 days followed by a dosage of 0·05 gramme every 8 hours for another 5 days is preferable, and if necessary it can be repeated in 14 days' time. Naturally every care must be taken to avoid dehydration or combat it if present in chronic as well as in acute cases.

(3) *Coincident Treatment.*

An initial dose of sodium or magnesium sulphate (2 drachms) is given as a routine and this should be followed in 2 hours by gentle colonic lavage, the bowel being washed out with 1 pint of warm saline solution (0·85 per cent.), using a well lubricated soft rubber catheter which is not inserted more than 2 to 3 inches into the rectum. Subsequently sulphaguanidine treatment is started. No further saline aperients are given, as any local bacteriostatic action excreted by the drug would tend to be decreased by increased fluidity of the stools and the accelerated rate of passage through the large bowel. Tinct. opii m.xv., or in severe cases morphia $\frac{1}{4}$ grain, may be administered and repeated if

deemed necessary when there is severe colicky abdominal pain, tenesmus or very frequent stools, *i.e.*, thirty or more in 24 hours. By slowing down the rate of peristalsis, opiates should tend to prolong the action of sulphaguanidine and so increase its local bacteriostatic effect. Heat in the form of hot-water bottles, antiphlogistine or turpentine stoups applied to the abdomen may be helpful for the first 24 hours before sulphaguanidine has exerted its maximal beneficial effect.

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subnormal temperatures, (*b*) profuse vomiting, (*c*) choleraic type of stool, (*d*) cramps, (*e*) rigors.

(iv) Dysentery in patients over 40 years of age, or in whom complications have supervened.

(v) Patients who develop acute bacillary dysentery during the course of some other illness.

(vi) Cases of chronic dysentery showing isolated ulcers or generalized inflammatory lesions of the colon resembling those seen in ulcerative colitis.

(5) *Clinical Experience with Sulphaguanidine.*

First impressions are sometimes important, but only if confirmed by subsequent experience. It happened that amongst the first cases treated were three patients with chronic dysentery with demonstrable ulcers in the colon. Two were cases of Flexner infection and had been under observation for 3 months; during which period repeated sigmoidoscopic examinations had shown that despite the use of every form of treatment then known, healing of the ulcers did not occur. In less than 14 days after sulphaguanidine treatment had commenced these ulcers were found on sigmoidoscopy to have healed, the bowels were acting normally and the stools showed no abnormal features. In the third case the dysentery had persisted for at least 6 months, during which period the bowels had acted six to eight times daily. Multiple ulcers were demonstrated on sigmoidoscopy and *B. dysenteriae* Shiga isolated. Treatment with sulphaguanidine was instituted a few days later and, within a fortnight, sigmoidoscopy revealed no evidence of ulceration, the bowel presenting a normal appearance; symptoms also permanently disappeared. In a wider investigation of some 500 cases, in which the drug was mainly reserved for severe infections and chronic cases, the value of sulphaguanidine therapy has been confirmed, the most striking feature being the immediate amelioration of symptoms which occurred virtually invariably as soon as the drug was exhibited. Shiga dysentery received special attention, and, as diagnosis was based on the bacteriological isolation of *B. dysenteriae* Shiga, the commencement of treatment was often delayed for 24 to 48 hours or longer on this account. Our preliminary report dealing with 135 Shiga infections has already been published (Fairley and Boyd, 1942), but as further data are not at present available the findings in this series will be recapitulated.

Acute Cases.—In a series of eighty-four recovered cases treated within the first 3 weeks, in which information contained in the case histories was sufficient to permit analysis, there was early well marked improvement manifested by an increased feeling of well being, rapid relief of abdominal pain and a decrease of abdominal symptoms. In uncomplicated cases an early reduction of temperature and pulse rate took place, normal being reached in most cases in from 1 to 3 days. There was also a speedy reduction in the number of stools and a

rapid disappearance of blood from the faeces, mucus disappearing some days later.

Analysis showed that the average time taken for the stools to reach two or less per day was 5·3 days when treatment was commenced within the first 5 days of illness, 5·4 days when it was commenced within the 6th to 10th day period and 5·0 days when it was commenced from the 11th to the 20th day. In the same three groups the average time taken for the stools to become formed were 7·7, 7·21 and 8·3 days respectively. Such a result can only be interpreted by assuming that sulphaguanidine was exerting a bacteriostatic or bacteriolytic action on dysentery organisms in the bowel wall.

Owing to the delay occasioned by waiting for a bacteriological diagnosis few cases in the 1 to 5 day series received sulphaguanidine before the 4th day. Had they been treated earlier, it is probable the results would have been even more satisfactory, as much of the damage done to the bowel in Shiga dysentery is done during this initial period. Two factors are concerned in the duration of the persistence of symptoms after the institution of sulphaguanidine treatment: the first is the control and elimination of *B. dysenteriae*, the second is healing of the colonic lesions produced prior to the establishment of effective treatment.

Subacute and Chronic Cases.—Nine of the twelve subacute or chronic Shiga infections were definitely cured with the standard course of treatment prescribed.

In the tenth case *B. dysenteriae* Shiga was isolated on the 9th and 38th days. Treatment commenced on the 41st day and during the next 5 days 96 grammes were given (an inadequate course). Relapse occurred. Sigmoidoscopy revealed a small red area on the second valve of Houston which bled on swabbing. On the 69th day a second course was started, 72 grammes being given. Thereafter the red raw area healed completely and the patient was discharged cured. These findings suggest that the initial treatment was inadequate, and that a full course in the first instance would probably have resulted in complete cure.

In the eleventh case treatment commenced on the 69th day. The course was inadequate and looseness of the bowels persisted. On sigmoidoscopy a few areas of redness and oedema were found on the summit of the valves which bled on swabbing. Cultures were negative. For some weeks the bowels acted four to six times daily. Healing here was incomplete, but no bacteriological evidence was obtained of persisting Shiga infection.

In the twelfth case treatment started on the 42nd day after the patient had relapsed. A complete course of treatment was given without controlling the diarrhoea. Data regarding the sigmoidoscopic and bacteriological investigations were not forthcoming and in the absence of this information treatment must be regarded as having failed.

Fatal Cases.—Five out of a series of over 500 cases receiving sulphaguanidine died.

The first two patients were moribund before treatment commenced and

did not survive long enough for the drug to exert any beneficial effect. Only 12 and 14 grammes of sulphaguanidine were given before death, which occurred within 24 hours in each instance. The third patient received very intensive treatment, but died from a cerebral thrombosis during convalescence. At autopsy the colon was shown to have completely healed ; chronic pyelonephrosis of the right kidney was also found. In the fourth and fifth cases information was lacking in the case records regarding both the quantity of sulphaguanidine administered and the duration of treatment. One had advanced chronic bilateral hydronephrosis and the other died of lobar pneumonia, which was diagnosed shortly after sulphaguanidine treatment commenced. All these fatal cases had complications and it is doubtful if any of them can be regarded as examples of the failure of sulphaguanidine therapy.

(E) OTHER SULPHONAMIDE COMPOUNDS.

The disadvantages of sulphaguanidine are its cost and the large dosage employed. Good as it is, future research may produce some sulphonamide equal or superior in anti-bacterial action and without these drawbacks. The first essential in the treatment of any disease like dysentery in which dehydration is liable to occur is that the sulphonamide used shall not cause renal blockage by the formation of hard concretions from crystals precipitated in the kidney or ureters.

Of the sulphonamide drugs which are well absorbed, sulphapyridine, sulphathiazole and sulphadiazine are prone to produce renal complications and cannot be recommended for this reason, even though they do exert a satisfactory bacteriostatic action on *B. dysenteriae* via either the alimentary tract or the blood stream. On the other hand, sulphanilamide and its acetyl derivative are both readily soluble, while MACARTNEY, LUXTON, SMITH, RAMSAY and GOLDMAN (1942) have shown that sulphamethazine and acetylsulphamethazine have a relatively high water solubility over a range of pH from 5·5 to 9·5.

Sulphanilamide is contra-indicated by its unpleasant effect, nausea and vomiting being well marked features when it is administered to cases of dysentery.

There should be little risk of renal damage with either sulphamethazine or acetylsulphamethazine and for this reason they are preferable for use in hot climates where the urinary secretion is low, or in diseases producing dehydration like dysentery and cholera provided, of course, they be found to exert appropriate anti-bacterial action.

Of the poorly absorbed drugs sulphasuxidine, which is a succinic acid derivative of sulphathiazole, alone needs consideration. It is sparingly soluble in water and poorly absorbed, not more than 5 per cent. of the ingested drug being excreted by the kidney. Only low concentrations are found in the blood

and its action is essentially in the alimentary tract. It drastically alters the bacterial flora, reduces growth of *B. coli* and anaerobic proteotype bacteria, while *in vitro* it exerts an anti-bacterial action on *B. dysenteriae* of Shiga, Flexner and Sonne type. Unfortunately, like sulphaguanidine the dosage is very considerable, being 0·25 gramme per kg. initially and approximately 0·04 gramme per kg. 4-hourly thereafter.

Both sulphamethazine and sulphasuxidine are at present under extensive clinical trial and the results in bacillary dysentery will be awaited with great interest.

Summary and Conclusions.

1. Bacilli causing dysentery in the Middle East included the six Flexner and three Boyd strains, as well as *B. dysenteriae* of Shiga, Schmitz and Sonne types.

2. *B. dysenteriae* Flexner II and VI were the two most common organisms encountered, *B. dysenteriae* Shiga being the third.

3. The variable sigmoidoscopic appearances in subacute and chronic dysentery and in carriers are described; certain of these have not previously been recorded.

4. Sigmoidoscopy plays a valuable role in the diagnosis of subacute and chronic dysentery, in the control of treatment and in detection of the carrier. Material collected by direct swabbing of the ulcers may yield positive findings where previous cultured investigations of the stools were negative.

5. The various clinical types of acute, subacute and chronic dysentery and post-dysenteric diarrhoea are described.

6. Complications which have not previously been recorded include (1) peripheral circulatory failure and (2) renal failure associated with azotaemia, oliguria, anuria and uraemia. Toxaemia and dehydration, and possibly haemoconcentration and changes in the concentration of serum proteins, are factors in their pathogenesis.

7. Chronic peritonitis with localized or more generalized effusion of fluid into the peritoneal cavity was also encountered. The condition is non-surgical and has to be differentiated from perforation with acute peritonitis.

8. Therapeutic results proved disappointing with polyvalent anti-dysenteric serum: serum sickness was a common complication.

9. Refined anti-dysenteric Shiga serum (1 c.c. = 20,000 I.U.) prepared by partial peptonization produced definite clinical improvement and stigmata of toxaemia decreased. Unfortunately the benefit was too often only temporary and improvement was not maintained.

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result was dramatic. I have been giving the drug in about a 3 per cent. solution (7 grammes in 7 ounces of water) for from 7 to 10 days. In this form sulphaguanidine is readily retained and is well tolerated.

Since then I have tried it out in a series of well-established and unmistakable cases of ulcerative colitis, especially of the regional form where the ulcerative process is confined to the sigmoid and rectum. There are three cases in particular, all of whom had been ill for a long time and had been subjected to numerous blood transfusions. The results at the time appeared to be rather dramatic, the blood and mucus have gradually disappeared from the stools and the patients have been restored to health: but it is really too early yet to say what the ultimate results may be in all cases. All that can be said at present is that it does not prevent relapses at some future date—it may be after a quiescent period of one year or longer, but the evidence is so far so encouraging as to justify further work on these lines.

Dr. H. J. Smyly: I thank Colonel FAIRLEY for his valuable and opportune paper. I have just returned from occupied China, where I have been working in Cheeloo University Hospital, Tsinan, Shantung. The hospital has been closed since 8th December, 1941. In the autumn Messrs. E. R. Squibb & Sons, New York, kindly sent me a generous quantity of sulphaguanidine for clinical trial. It arrived in December, but I was allowed by the Japanese Military Authority to take delivery of the drug from the Post Office. As our hospital was closed I arranged with my former colleague, Dr. M. L. SUN, now attached to Tsinan Municipal Hospital, to collaborate with me in a clinical trial on cases of dysentery during the summer of 1942. Before I left he was able to give me notes on ten cases and I had in addition five cases in Europeans of our interned community.

The cases selected were :—

1. Those of less than 24 hours' duration with typical cell exudate of bacillary dysentery under the microscope;
2. Children under 10 years old;
3. Severe cases with over fifteen stools in 24 hours;
4. Chronic cases of over 2 months' duration.

This excludes mild cases of a few days' duration which readily clear up with simple treatment.

Diagnosis was made by examination of the stool with special attention to microscopic examination.

A positive diagnosis was made on finding a typical exudate of polymorphonuclear leucocytes and macrophages along with red corpuscles in a mucous dysenteric stool. Cultures were made from stools in ten cases, in most of them twice, but owing to war restrictions culture media were defective, and all cultures from these and other cases at this time were negative.

Dosage was that recommended by previous workers :—the initial dose : 0·1 gramme per kg. body weight, followed by 0·05 gramme per kg. every 4 hours until stools are under five a day ; then 0·05 gramme per kg. every 8 hours for at least 3 days and until stools have become normal and cultures negative for dysentery bacilli.* Of the fifteen cases fourteen were acute and one chronic.

Ages ranged from 1 to 59 years. The duration of symptoms before treatment was 1 day or less in eight cases ; in others 2, 3 and 7 days. All but one (treated abortively) had typical symptoms of dysentery. Temperatures were above normal, in one case over 102° F. The number of stools in 24 hours was from ten to thirty ; cell exudate was typical.

In treatment the usual precautions were followed. Patients were put at rest in bed ; liquid diet with abundant fluid was prescribed. Sulphates were not given during the acute phase, but when the motions became few, small doses were given to keep the faeces fluid until all leucocytes had disappeared from them. Results were definite. Fourteen acute cases improved rapidly and were clinically well in from 2 to 4 days. One chronic case showed no improvement. One of the first cases was in myself, so that I may say that I have inside information. I was taken ill one evening, passed three loose stools during the night and six the following morning. I felt extremely ill and prostrated, with nausea, retching, and anorexia. At 11 a.m. a bloody mucous stool was passed which showed with the microscope a typical bacillary dysentery exudate. Sulphaguanidine 5 grammes was taken at 1.30 p.m., and 2·5 grammes 4-hourly thereafter. Diarrhoea rapidly diminished and all subsequent stools were liquid faecal. On the morning of the 3rd day the microscope showed degenerated leucocytes but no fresh cellular exudate. This unusual finding indicates an abrupt cessation of acute inflammation, only the residue of previous exudate being passed. By the next day only a few ghost cells could be found. I returned to work a week from the onset, but another week passed before the sense of normal fitness was restored. This is a typical case. Another patient, a foreign lady, age 32, improved rapidly but returned too soon to normal diet and developed a persistent diarrhoea with liquid faeces free from cells. Rest, bland diet and bismuth were followed after 2 weeks by complete recovery. She had had a " summer diarrhoea " some years before, which lasted till the autumn.

One of my colleagues has three little girls aged respectively 10, 7 and 2. All developed dysentery one after the other. The first two were rapidly cured with sulphaguanidine. The third case was as follows : She woke in the morning with abdominal pain, had two loose stools before breakfast, was given castor oil 1 drachm. By noon her temperature was 100° F. and she still had abdominal pain. Without sending a stool specimen for microscopic examination she was given sulphaguanidine 2 grammes at 2 p.m. ; she had two more stools

* LONG, P. H. (1941). *Canad. med. Ass. J.*, 44, 217.

that day. By evening fever and pain subsided. Sulphaguanidine was continued for 3 days: on the 2nd day one stool only; examination showed degenerated leucocytes. This may be regarded as an aborted attack.

The chronic case was in a Chinese male, aged 26, under the care of Dr. M. L. Sun, with whom I saw the patient in consultation. His dysentery began 2 years before. He was passing two to four fluid stools daily, containing a little mucus. The microscope showed leucocytes and red corpuscles with a few macrophages. Stool cultures were negative, and our sigmoidoscope had been sequestrated. He was given sulphaguanidine, 6 grammes daily for 9 days, and then 15 grammes daily for 8 days, in all 174 grammes in 17 days. In spite of this dosage, which is both larger and more prolonged than is advised, he showed no improvement.

No untoward symptoms nor urinary abnormality occurred in any of these cases.

Colonel E. R. Boland: My experience of bacillary dysentery is limited to the last 2½ years in the Middle East. I had the advantage of a year's experience without sulphaguanidine treatment and 1½ years with sulphaguanidine, and I am still preserving an open mind as to the exact estimation of this form of therapy. It is fair to say that at a meeting held in Cairo in April, 1942, of all the officers in charge of Medical Divisions in the Middle East, my views were more sceptical than those of the majority of my colleagues, but I still think there has been no sufficiently controlled series of cases to make it possible to estimate the exact effect since dysentery in the Middle East is comparatively so mild, the mortality so low and almost spontaneous recovery so general that it is impossible to forecast accurately the course of any individual case.

The only possible method of control is to have a large series in which alternate cases are treated with or without sulphaguanidine and compare the periods of hospitalization necessary in the two groups. The mortality rate is so low that it would be unlikely to afford much information. I never had a sufficient supply of sulphaguanidine for this method of control to be tried. The only attempt at control I was able to make was to compare the course of the disease in sick Italian prisoners of war and British patients. In the hospital where I worked there were beds for 1,200 British and 1,000 Italians. For each British patient treated with sulphaguanidine, it is necessary to fill up a rather complicated form to show the progress and effect of the treatment; I got the Italian medical officers to fill up the same progress forms for all their cases of dysentery which were not given sulphaguanidine. Before leaving Egypt I had collected about 200 forms of each group, but unfortunately left them behind as I came away rather unexpectedly. A preliminary study of the forms suggested that it would be very difficult to detect which cases had had sulphaguanidine and which had not, since so many apparently acute cases improved suddenly with only saline treatment.

A case which I saw about a month ago well illustrated the difficulty of estimating the value of this therapy. A nursing sister was admitted with all the characteristic features of the severest form of dysentery. There was repeated vomiting, she had nearly forty stools in the 24 hours with much pain. The pathologist reported that there were large shreds and filaments of mucous membrane in the stools; her temperature was 104° F. and she had a rapid thready pulse and marked mental symptoms. I gave her 100,000 units of Shiga serum and ordered her to have sulphaguanidine. I was not able to see her the next day as I was away but I went to see her on the 3rd day expecting to find her still very ill. Instead I found her almost completely well with no diarrhoea, a clean tongue and normal pulse and temperature. I found that her recovery took place the day after I saw her. You might attribute this rather remarkable recovery to the anti-Shiga serum I had given her and you would be wrong because it turned out she had a Flexner infection; you might attribute it to the effects of sulphaguanidine and again you would be wrong because, owing to a mistake, she was never given the drug ordered. This case is an extreme example of that large group of cases admitted with twenty or thirty stools a day which settle down in a few days with rest in bed and routine treatment. In many such cases, the credit is now given to sulphaguanidine when it should really be given to Nature. I do not want to suggest that I do not think sulphaguanidine is of value but only wish to point out that one is not yet in a position to determine its exact value.

An encouraging feature is the effect in some cases of chronic dysentery controlled by the length of history in which the recovery appears to have been dramatic after sulphaguanidine. In the absence of a severe epidemic, it is too early to say that one has a treatment as specific for dysentery, as say M. & B. 693 for meningococcal meningitis, and a controlled series would probably show that the actual duration of treatment is only cut down by 2 or 3 days at most. Ulcerative colitis has been referred to this afternoon. We had two cases of ulcerative colitis in which the disease was contracted a year or two before going to Egypt and in neither case was there any relief with sulphaguanidine.

Dr. D. Riding: One thing I would say after 15 years' experience in Egypt and the Sudan is that bacillary dysentery seems to affect people during their first 2 or 3 years residence in the Middle East. After that they may get attacks of "gippy tummy" but seldom of frank dysentery. As the troops have been out there 2 years it will be important, in the absence of controls, to take into account this natural decrease in the incidence of dysentery, when assessing the value of these newer drugs.

Dr. A. Felix asked Colonel FAIRLEY if he had had personal experience of the bacteriophage treatment of bacillary dysentery. Dr. A. COMPTON, of Alexandria, had been advocating this treatment for many years and recently published figures

showing the case fatality rates from dysentery in Alexandria, in Cairo and in the rest of Egypt over a period of 12 years.* COMPTON concluded that the case fatality rate had fallen only in Alexandria and was now much lower there than anywhere else in Egypt, and he attributed this to the introduction of the bacteriophage treatment in the municipal hospitals and clinics. Colonel FAIRLEY might perhaps be in a position to give first-hand information on this point.

Colonel Fairley (in reply): I was extremely interested in Dr. CRUICKSHANK's remarks about the higher proportion of positive cultures on the improved desoxycholic acid medium for *B. dysenteriae*. We got positive cultures from swabbings of ulcers during sigmoidoscopy when examination of the ordinary stools failed. With an improvement in cultural technique such as this, sigmoidoscopy might become less important in detecting chronic and carrier cases. The Flexner cases in our series did well with sulphaguanidine and so did the few cases of Sonne, but Sonne is an uncommon infection in troops in the Middle East and only a few observations were made.

With regard to Dr. SMYLY's case which did not respond, possibly it was ulcerative colitis. In the Middle East we treated a number of people who gave a history of ulcerative colitis or the passage of blood and slime in the stools before coming out there. Investigation failed to reveal dysentery bacilli and the sigmoidoscopic appearances were typical of ulcerative colitis. In these cases we got no satisfactory results from sulphaguanidine treatment.

I take it that Sir PHILIP MANSON-BAHR's remarks refer to chronic ulcerative colitis and to chronic bacillary dysentery in which dysentery bacilli can still be isolated. It would be impracticable to treat patients with acute bacillary dysentery by medicinal enemas, as the bowel would not tolerate any interference of that sort in the early phases of the disease. The idea of Dr. MARSHALL, when introducing sulphaguanidine, was to get a uniform saturation of the faeces by oral administration of an intestinal antiseptic which in large dosage is poorly absorbed. I do not know whether you can get better concentration over the whole surface of the colon by the rectal rather than the oral route, but it may be you can. Apparently absorption of sulphaguanidine takes place in the small not in the large bowel.

In regard to Colonel BOLAND's remarks, Colonel BOYD and I were both disappointed that we were not able to do a control series of cases, but owing to the limited supply of sulphaguanidine and the absence of an epidemic in which Shiga's bacillus predominated, it was not feasible to do so. In every patient with clinical dysentery in the Middle East there are about twelve bacteriological possibilities, and for purposes of accurate comparison you must diagnose every case by isolating the causative organisms. This means that a very large series has to be investigated. Further, considerable damage is being done to the

* COMPTON, A. (1942). *Brit. med. J.*, 1, 719.

bowel while waiting for a bacteriological diagnosis. The value of our observations is that our series only includes proved cases of Shiga dysentery, most of which were really severe infections. Judging by our previous disappointing experience in severe Shiga infections treated on approved lines with salines and anti-dysenteric Shiga serum of high potency, and by the subsequent extremely good results with sulphaguanidine treatment in similar cases, I think a very definite case has been made for its efficacy in both chronic and acute Shiga dysentery. I cannot imagine a specific drug being expected to do more than sulphaguanidine has done in this series, especially considering that the bowel was extensively damaged before treatment was commenced. I agree with Colonel BOLAND with regard to the mildness of much of the dysentery in the Middle East, and this was emphasized in my introductory remarks when I said most cases of Flexner dysentery get well with rest, fluids and proper diet. Flexner dysentery in the Army is very rarely fatal. In one of our Australian hospitals 600 dysentery patients were treated including thirteen Shiga cases without mortality. I have seen hundreds of cases of Flexner dysentery in New Guinea in which they have had similar treatment (with perhaps a little magnesium sulphate in addition) and none have died. I agree that in the Army we could not assess the value of any specific drug in non-Shiga dysentery on the basis of mortality rate alone.

In the present campaign in the Middle East the distribution of different strains of *B. dysenteriae* has remained pretty constant, Shiga accounting for about 10 per cent. of cases. Nothing comparable with the last war has been experienced in the Middle East when there was as much as 60 per cent. Shiga bacillus isolated in an epidemic. Under such circumstances it would be possible by a controlled series of observations to determine what the life-saving effectiveness of sulphaguanidine is, as well as the effect on the period of hospitalization and convalescence. But we are at war, and, being convinced that sulphaguanidine is effective, I, for one, would not be prepared to advocate a large-scale experiment in an epidemic of Shiga dysentery and have a number of troops dying or incapacitated for long periods who might otherwise have recovered. From this viewpoint it is a great pity we could not have done the controlled experiment earlier before we had formed definite impressions on what the drug could do.

Regarding the question of people who have been resident in the Tropics or Middle East for a year or two and do not get much dysentery afterwards, that is a universal experience. In certain countries, when fresh troops come into a new area we know the first thing to do is to get ready to receive dysentery cases. Whenever a regiment from Great Britain goes into the Middle East we know that the dysentery incidence is liable to rise, and it is the same thing in New Guinea when troops from Australia go there. Afterwards they get "salted" and this sort of thing does not happen—at least not to anything like the same extent.

In regard to bacteriophage, there was no significant difference between cases treated with bacteriophage and cases which were treated with magnesium sulphate, but as no Shiga cases were included in either series, and as both series of cases were only small ones, the results cannot be regarded as having much value one way or the other. Neither Colonel BOYD nor I was ever impressed with bacteriophage therapy either in India or in the Middle East, and we are frankly sceptical about the enthusiastic claims which have been and are being made on its behalf.

With regard to amoebic dysentery, I have seen amoebic ulcers heal during sulphaguanidine therapy, but the condition has relapsed shortly after cessation of treatment. It appears unlikely that sulphaguanidine will be found to exert any specific anti-amoebic action, though it might assist healing of amoebic ulceration if a secondary bacterial agent was implicated.

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COMMUNICATIONS.

CHILD MORTALITY IN LAGOS, NIGERIA.

BY

E. C. SMITH, M.D.*

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This paper presents an analysis of 500 postmortems, undertaken with a view to obtaining data in connection with the mortality of the children in Lagos.

By arrangement with the Medical Officer of Health, an autopsy was, where possible, carried out in the case of every child up to 3 years of age who had died without having been attended by a doctor and whose death therefore had not been medically certified. Many cases, owing to the prolonged interval which had elapsed before notification, were not considered suitable for examination and the number autopsied represents approximately 17 per cent. of the total death rate among the children in the age group specified.

The postmortem examination was made as complete as possible and the technique was similar to that prescribed by Barnard (1935) for the London County Council Laboratories. Routine paraffin sections were prepared from the liver, kidneys, lungs and spleen and frozen sections (for fat staining) were made from the liver, kidneys and heart muscle in each case. Routine smears, taken from the brain and spleen, were stained for parasites. The alimentary tract was examined for helminths and a count was made when necessary.

General Statistics.

Of the 500 autopsies performed, 259 were males and 241 were females. The monthly postmortem average as compared with the monthly average of total deaths for the period under consideration (1937-40) is shown in Table I.

* I am indebted to the Director of Medical Services for permission to publish, to Dr. Oluwole, Medical Officer of Health, for his kind co-operation, to Col. W. F. Harvey, Edinburgh, for histological reports and to members of the Staff of the Institute for their help and criticisms.

Mr. J. E. Knight, Laboratory Superintendent, is responsible for the illustrations.

TABLE I.

MONTHLY AVERAGES 1937-1940. POSTMORTEMS AND TOTAL DEATHS.

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Post-mortem averages	10	10·33	7·66	10·33	14·66	11	28	23	18·66	14	7·66	9·66
Total deaths averages	71	67	73·5	40	76·5	73	105	112	104	81·5	64	78

The mortality was highest during the months of July, August and September. (See graph.)

An analysis of the age groups is given in Table II.

TABLE II.

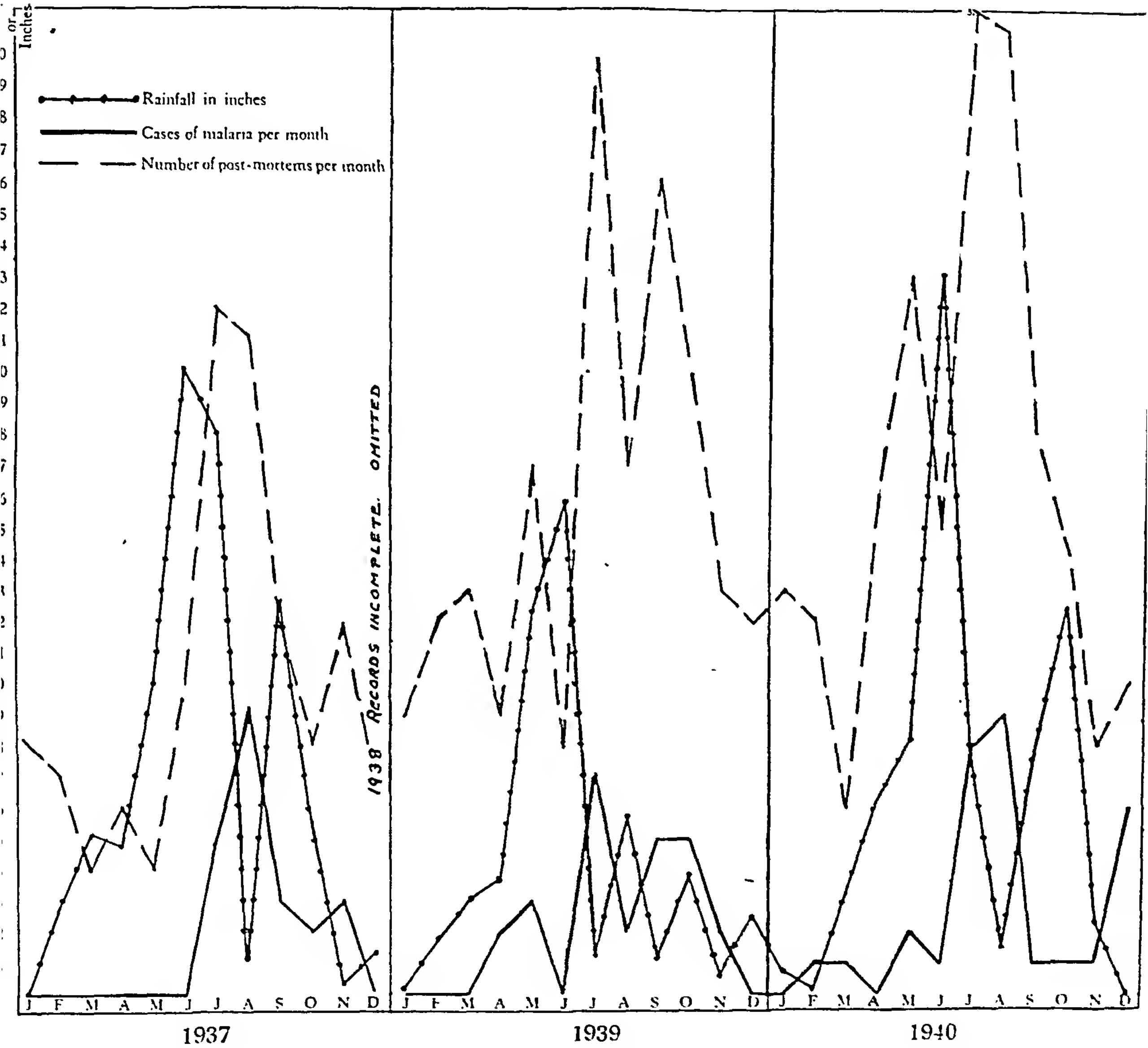
AGE GROUPS.

Age	Number of deaths.	Percentage.
0–6 months	181	36·2
7–12 months	112	22·4
13 months–2 years	137	27·4
25 months–3 years	70	14·0

SUMMARY OF POSTMORTEM FINDINGS.

	Number of Cases.	*Percentage.*
Respiratory diseases	343	68·6
Malaria	72	14·4
Diseases of alimentary tract	20	4·0
Meningitis	18	3·6
Anaemia	14	2·8
Malnutrition (faulty diet) and/or starvation	14	2·8
Congenital group	8	1·6
Miscellaneous	11	2·2
Total	500	

Clinical histories were unobtainable and the scanty information gleaned from parents or relatives was generally to the effect that the child had cough and fever, or convulsions and fever, or looseness of bowel, for a variable period prior to death.



GRAPH OF RAINFALL, MALARIA INCIDENCE AND NUMBER OF POSTMORTEMS PER MONTH.

Information as to the age of the child was again often inaccurate but an approximate check was made in all cases from a consideration of the state of the dentition and of the body length. FISCHER (1922).

Disease Groups.

RESPIRATORY DISEASES.

The 343 cases comprising this group were composed of the following :—

Bronchopneumonia ...	276	Pleurisy	3
Lobar pneumonia ...	26	Diphtheria	1
Pulmonary tuberculosis ...	32	Empyema	1
Abscess of lung	3	Gangrene of the lung ...	1

Of the 276 cases in which bronchopneumonia was considered to be the primary cause of death, eighteen were associated with malaria, fifteen with abdominal syndrome, eleven with sicklaemia and seven with pleurisy.

Bronchopneumonia played a contributory part in all of the 500 cases examined; in no case was it possible, after histological examination, to state that the lungs were normal. No evidence of the passage of helminth larvae was found.

The term abdominal syndrome is used here to signify that the bronchopneumonia was associated with certain visceral lesions suggestive of typhoid. The condition is frequently encountered in young adults and attention was first drawn to it by Dr. J. Cauchi, when Senior Health Officer for Lagos.

The abdominal organs affected include the intestines, spleen and mesenteric lymph nodes.

Spleen.—The spleen is enlarged and intensely congested. It is soft in consistency and in gross section presents a darkly coloured pulp set with enlarged oedematous-looking Malpighian bodies.

Perisplenitis may be present.

Intestines.—The changes in the intestines are the most marked and constant feature of the syndrome and involve the lymphoid tissue of the caecum, ascending colon and proximal few feet of the ileum.

The Peyer's patches are swollen and present an irregularly pitted surface resembling that of an inflamed tonsil. Ulceration is unusual but necrosis indicated by patchy bile staining may be present.

The scattered lymph follicles show similar changes and are usually surrounded by inflammatory haloes.

The mesenteric lymph nodes are swollen and congested. In cut surface the follicles are defined clearly against the congested background.

The thymus was enlarged in all these cases. Whether this enlargement is related to the condition under consideration or not, is difficult to say since an analysis of the 500 autopsies revealed a considerable variation in the size of the organ. Thus: in three cases it was absent, in 185 (37 per cent.) it was atrophic. The term atrophic is used to signify that the organ was just visible as a lobed structure.

In 229 instances it was regarded as being of normal size and weighed 10 to 20 grammes. In eighty-three cases (16·6 per cent.) the organ was enlarged and varied in weight from 25 to 50 grammes. Congestion was noted in forty-two of these. FISCHER (1922) states that the weight of the thymus is 17 grammes at 1 year and 23 grammes at 3 years.

YOUNG (1934) in the Nigerian *Report of Medical and Health Services for 1933*, refers to the exclusion, on bacteriological grounds, of typhoid infection in these cases and mentions the isolation of strains allied to *B. lactis aerogenes* and of the Friedländer bacillus.

Types of Bronchopneumonia.

These may be grouped on the combined naked eye and histological findings as follows :—

(1) *Haemorrhagic.*—In these, congestion, subpleural petechiae and haemorrhagic areas, some infarct-like in appearance, were found.

(2) *Bronchiolitic.*—In this type the inflammatory reaction was condensed around the bronchioles. The histological appearance in general resembled that portrayed by HUBBLE *et al.* (1941) in their cases of acute bronchiolitis in children.

(3) *Interstitial.*—In this form the lungs appeared denser than normal and histologically the alveolar walls were seen to be diffusely infiltrated with inflammatory cells mainly of the mononuclear type. Fibrosis was present to a varying degree together with some periateritis.

Of interest are certain cases, eight in number, in which scattered groups of irregularly formed large giant cells of the foreign body type were seen.

The giant cells were usually in clusters and surrounded sharply defined bodies structurally suggestive of cellulose or vegetable matter. Similar bodies were present within some of the giant cells and in the bronchioles.

It seems probable that the material was vegetable matter which had become inhaled during attempts at feeding or during the administration of native medicine. The method of infant feeding practised among the uneducated Lagosians has been described by WHITBOURNE (1930) and is quoted in full.

> " An infant is held, in the mother's lap with its head thrown back. The mother's right hand is hollowed to form a cup containing the water, the back of the hand is pressed firmly against the nostrils and the fluid poured into the mouth is swallowed amidst gasps and screams.
>
> Agbo, and later on agidi and corn flour are given in this way." . . . " Agbo is the generic name for decoctions of herbs of varying strength and composition."

In the earlier stages of the investigation blood agar plate cultures were made from the lungs but this practice had to be abandoned on account of gross contamination. No attempt was made to isolate a virus owing to the unfavourable

conditions. In a number of cases sections were stained with Giemsa for the presence of cytoplasmic inclusions. None were found. In 382 cases a portion of the lung was emulsified in saline and inoculated into white mice for the purpose of isolating pneumococci. These were isolated 135 times (34·4 per cent.) and the results of typing are shown in Table III.

It will be seen that Type 6 is predominant (45 per cent.) and that Types 1, 2 and 3 are rare.

These findings are in agreement with those of DAVIDSON (1938). As to whether the pneumococci isolated have any aetiological significance or not it is difficult to say. In only one instance were two types of pneumococcus (T 6 and T 21) isolated from the same lung.

Table III includes fourteen cases of lobar pneumonia. The types of pneumococcus isolated being as follows :—

T 4 and T 19	... ...	3 cases each.
T 8 and T 18	... ...	2 ,, ,,
T 6, T 7, T 20 and T 24...		1 case ,,

TABLE III.

Type	1	2	3	6	7	8	9	11	13	14	17	18	19	20	21	22	24	27	28	29
Number of cases	2	2	6	61	1	4	3	2	2	5	1	12	20	1	3	6	1	1	1	1

Organisms other than pneumococci were isolated as tabulated.

Streptococci, non-haemolytic ...	... ...	13
,, haemolytic	... ...	9
,, *viridans* type	... ...	3
Gram-negative haemolytic bacilli of the *B. coli* group		8
Gram-negative capsulated diplococci (Friedländer)...		5
Staphylococcus aureus ...	... ...	3
B. pyocyaneus ...	... ...	1

The cases from which *S. aureus* was isolated were of the lobar type. A pleural effusion was present in one and a culture of the fluid was positive for *S. aureus*. Abscess formation and empyema were present in a second case and here also, culture of the abscess material and of the empyema fluid were positive for *S. aureus*. It may be that this organism is a more frequent cause of pneumonia of children in West Africa than is generally recognized ; that the resultant inflammation may be lobar in type has been noted by SMITH (1935). HUGHES (1938) refers to the frequency with which staphylococcal pneumonia occurs in Singapore and stresses its association with abscess formation.

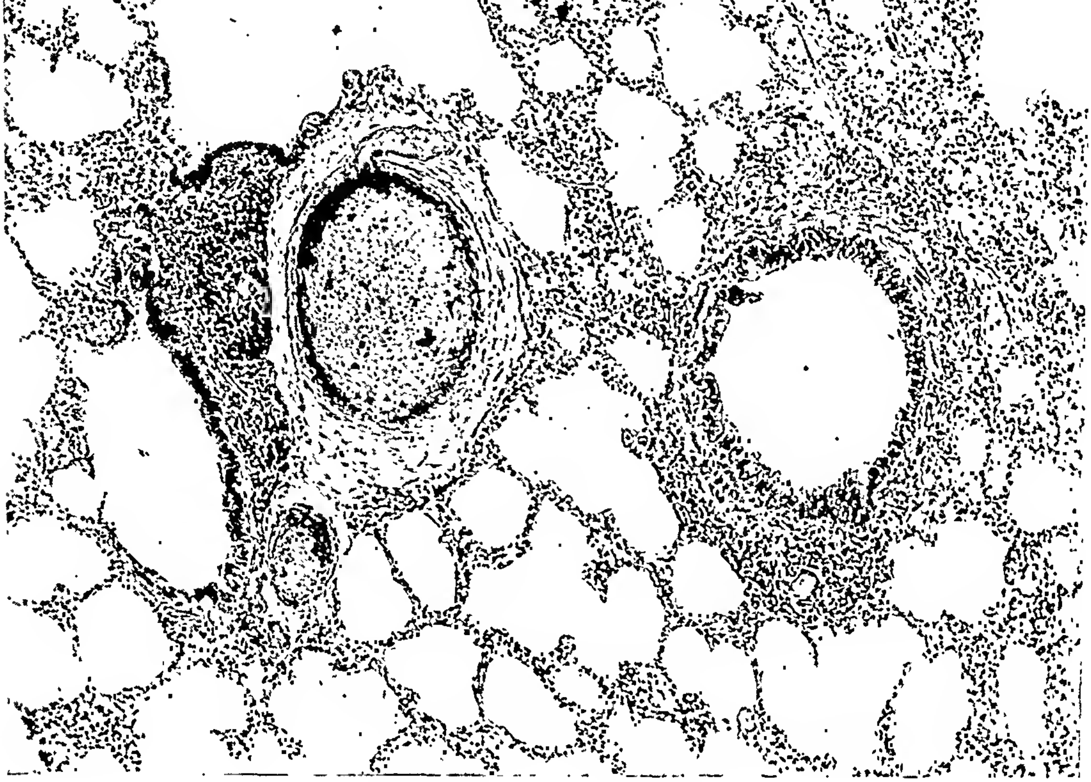


FIG. 1.—Bronchopneumonia.
Mild degree of interstitial infiltration with condensation of the reaction around the bronchioles ($\times 100$).

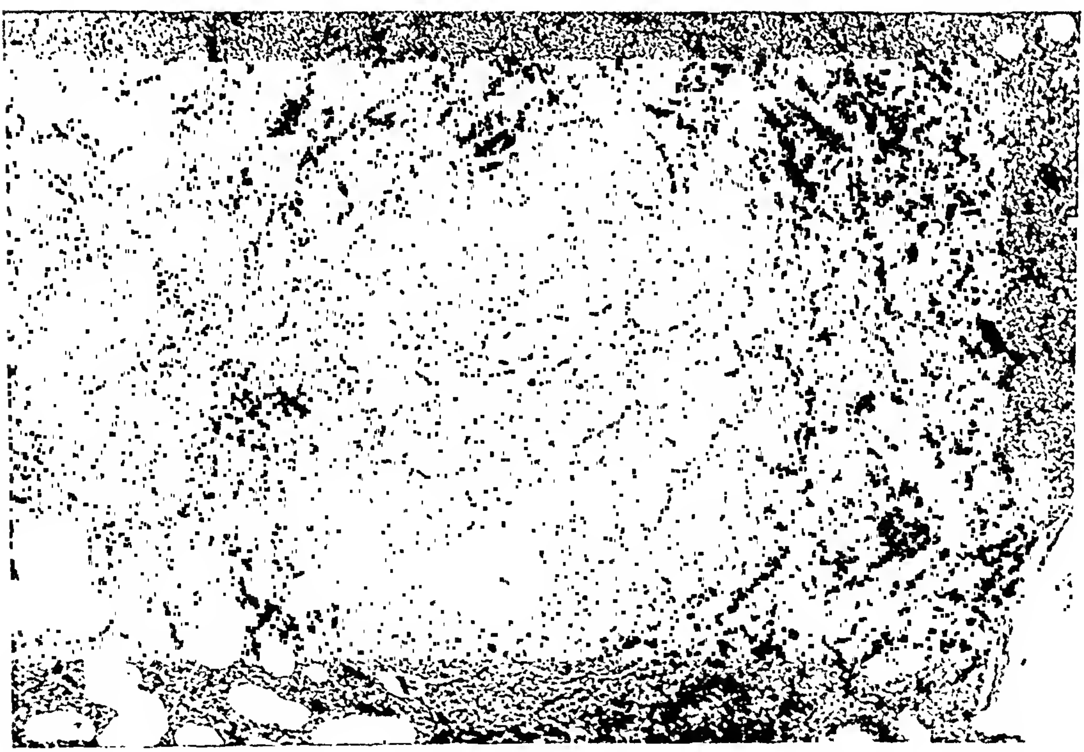


FIG. 2.—A case of lobar pneumonia in which commencing liquefaction is apparent ($\times$ *c.* 40).

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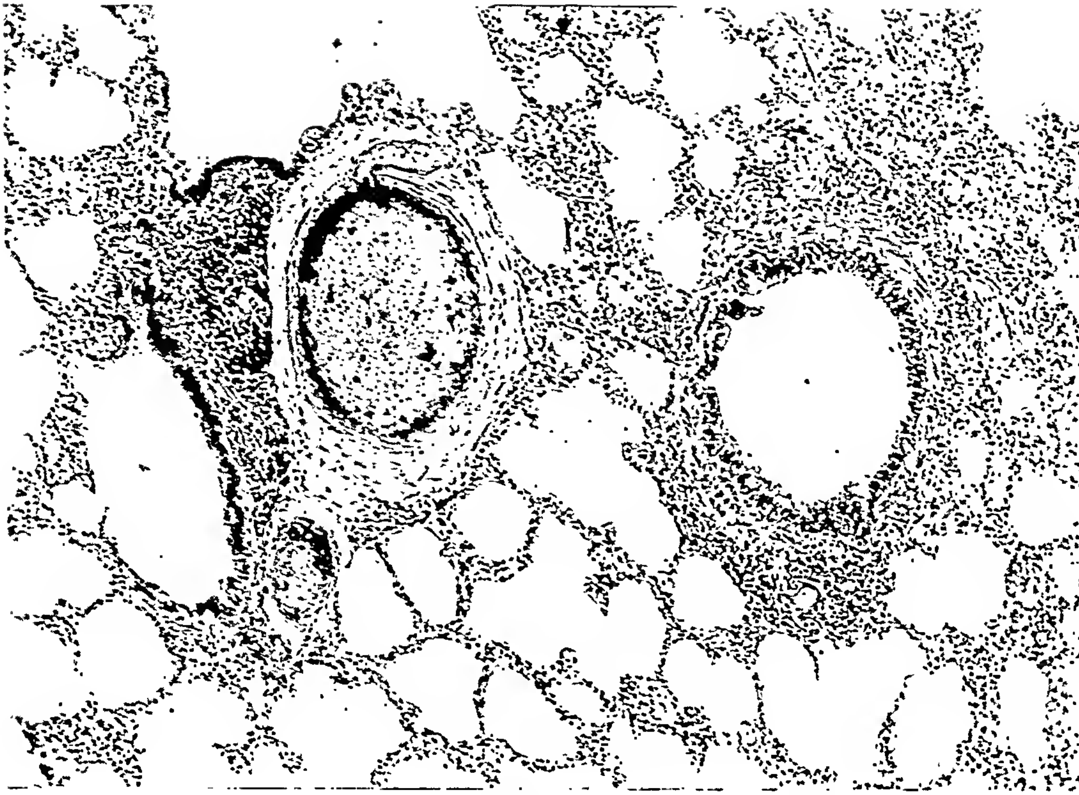

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Mild degree of interstitial infiltration with condensation of the reaction around the bronchioles (× 100).

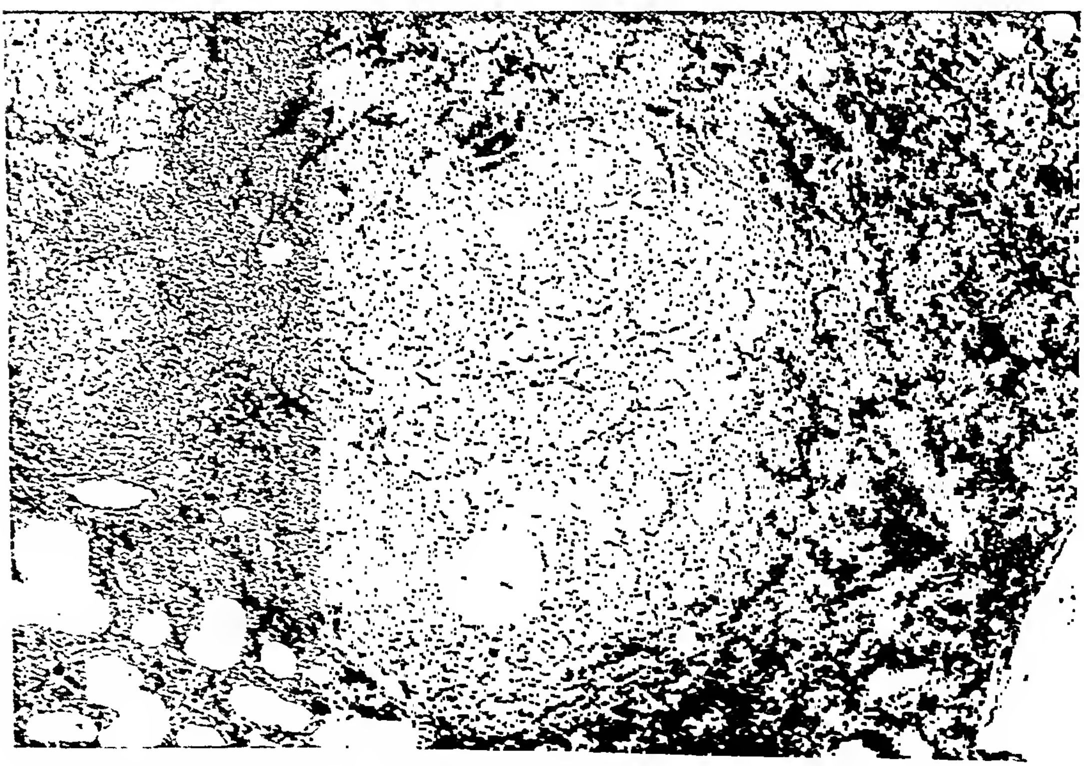


FIG. 2.—A case of lobar pneumonia in which commencing liquefaction is apparent (× c. 40).

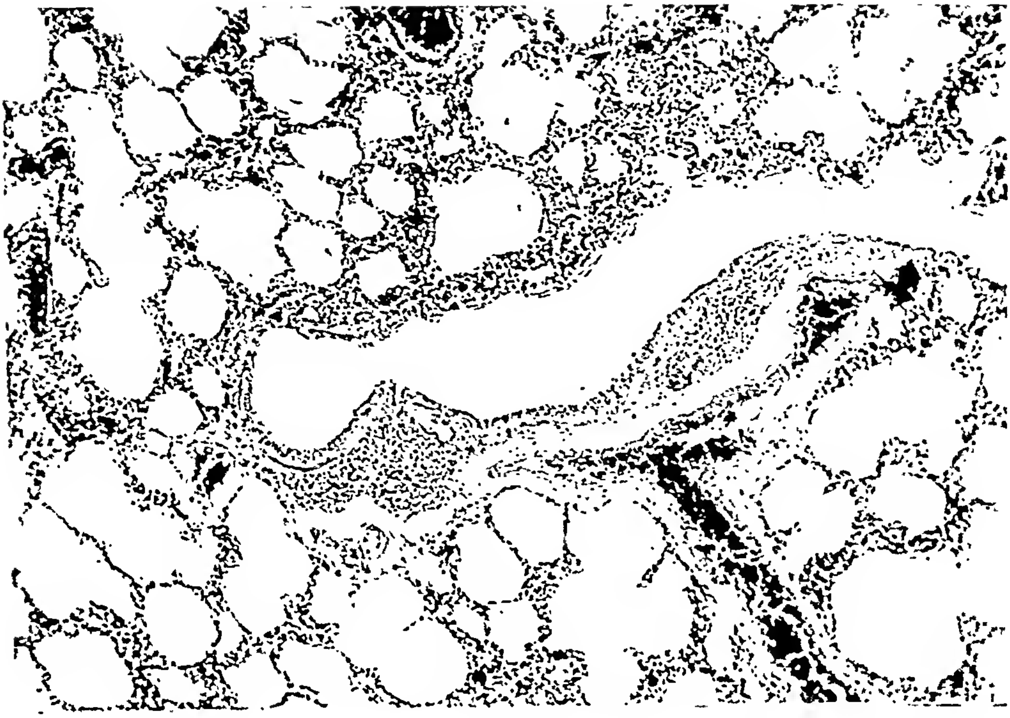


Fig. 3.

Fig. 4.—Bronchopneumonia of the interstitial type with commencing abscess formation, possibly within the lymphatics, in the wall of a bronchiole.
The illustration above shows two minute abscesses (× c. 70).
In the lower illustration an abscess is seen infiltrating the muscularis mucosae (× c. 160).

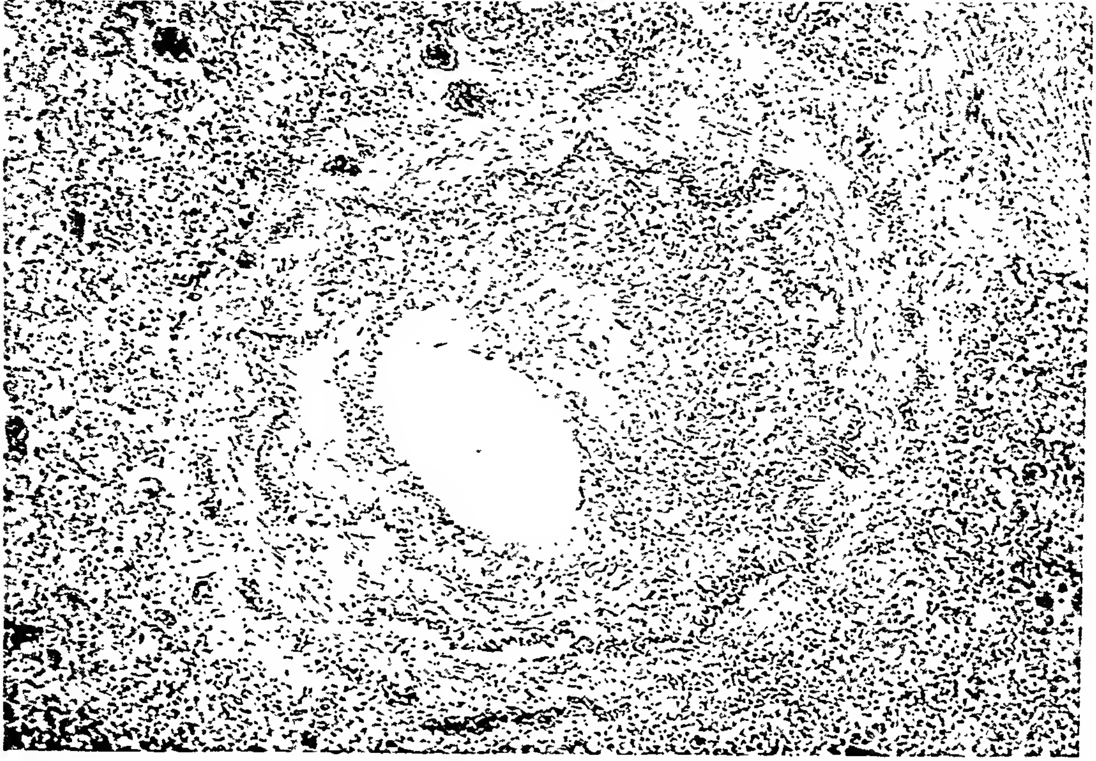


FIG. 5.—Bronchopneumonia. Organisation of the exudate within the bronchiole (× c 85).

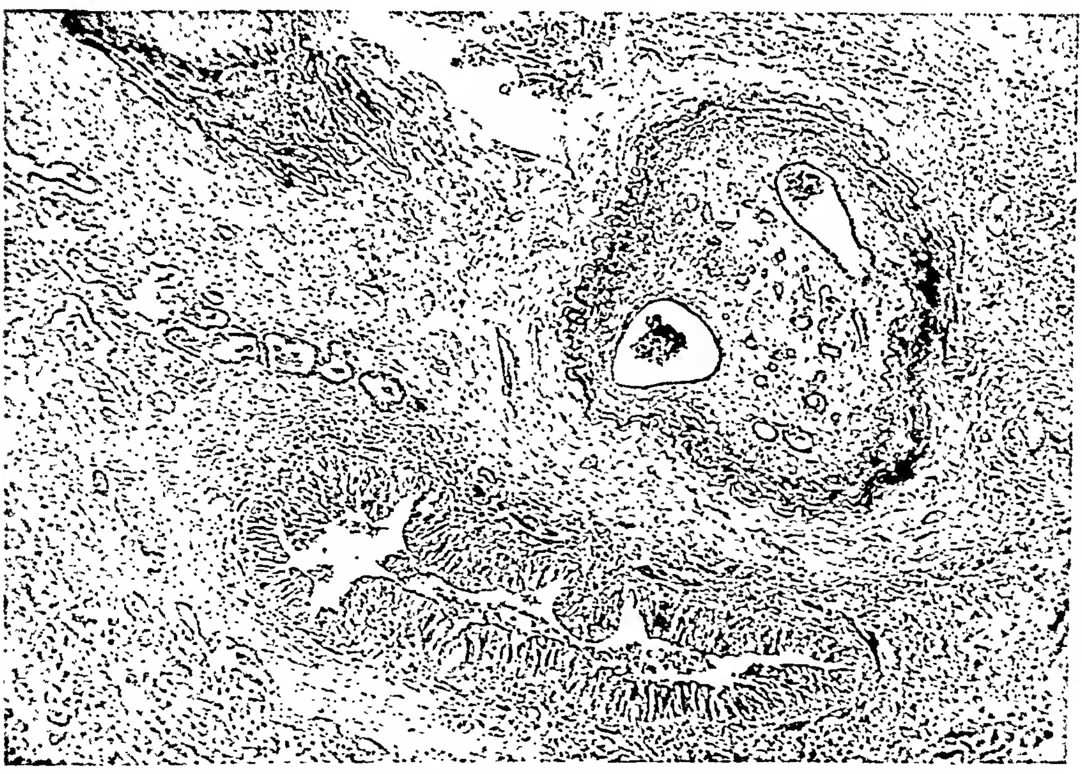


FIG. 6.—Section through the margin of an infarcted area from a case of lobar pneumonia in a male infant aged 7 months. A pulmonary vessel has undergone organisation with canalization. Below and to the left are collapsed pulmonary alveoli lined with cubical epithelium (× c. 85).

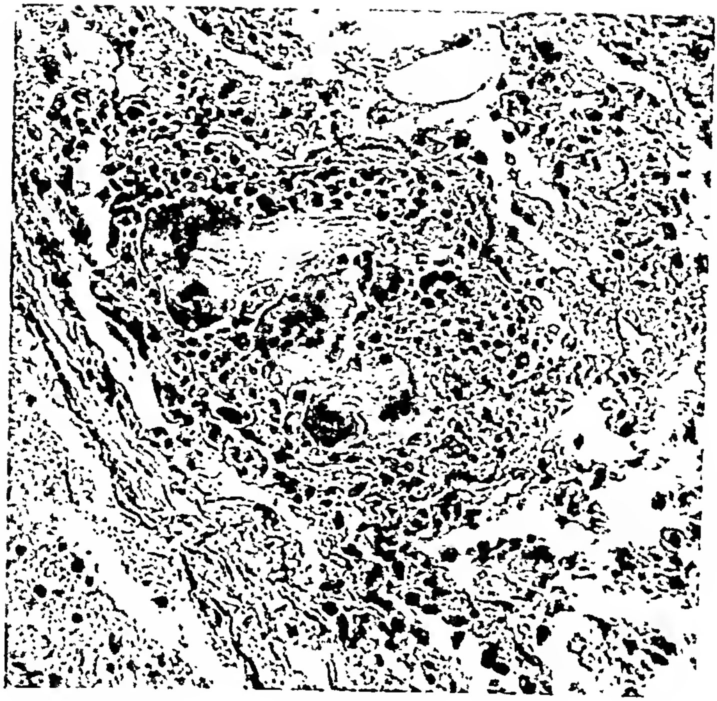


FIG. 7.

FIGS. 7 and 8 —Bronchopneumonia associated with the inhalation of extraneous matter. The illustrations show the nature of the reaction and the presence of foreign bodies. (Fig. 7 × *c.* 200 ; Fig. 8 × *c.* 250).

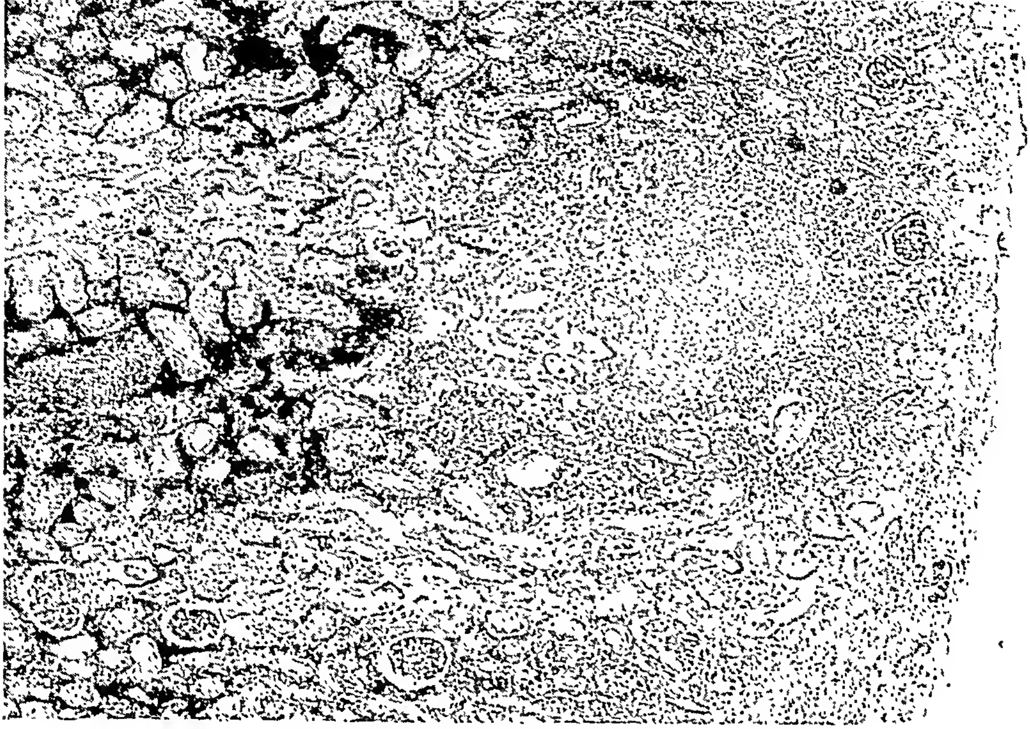


Fig. 9.

Figs. 9 and 10.—Tuberculous lesions in kidney and spleen.

Case of pulmonary tuberculosis with miliary spread. The lesions are of the type of infarct necrosis without the formation of tubercles or giant cells. Acid-fast bacilli were numerous (Fig. 9 × c. 65; Fig. 10 × c. 40).

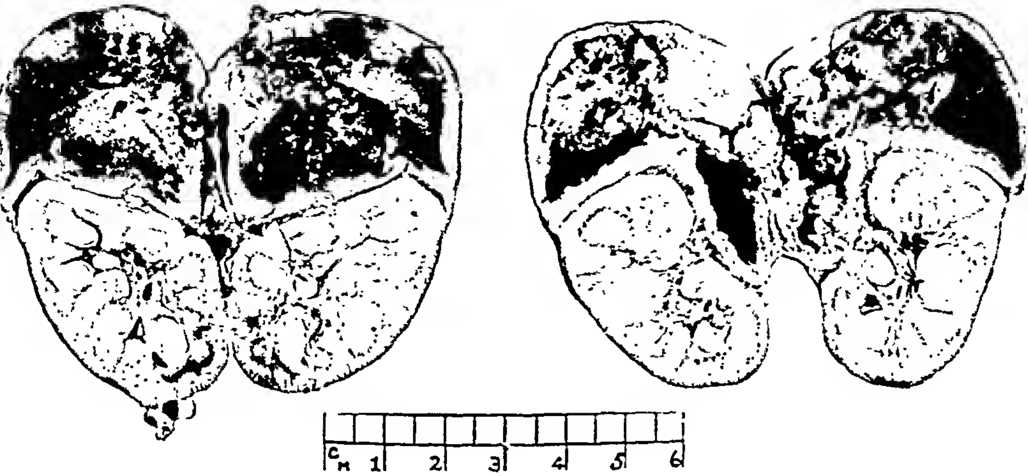


FIG. 11.—Bilateral suprarenal haematoma in a male infant aged 2 weeks.

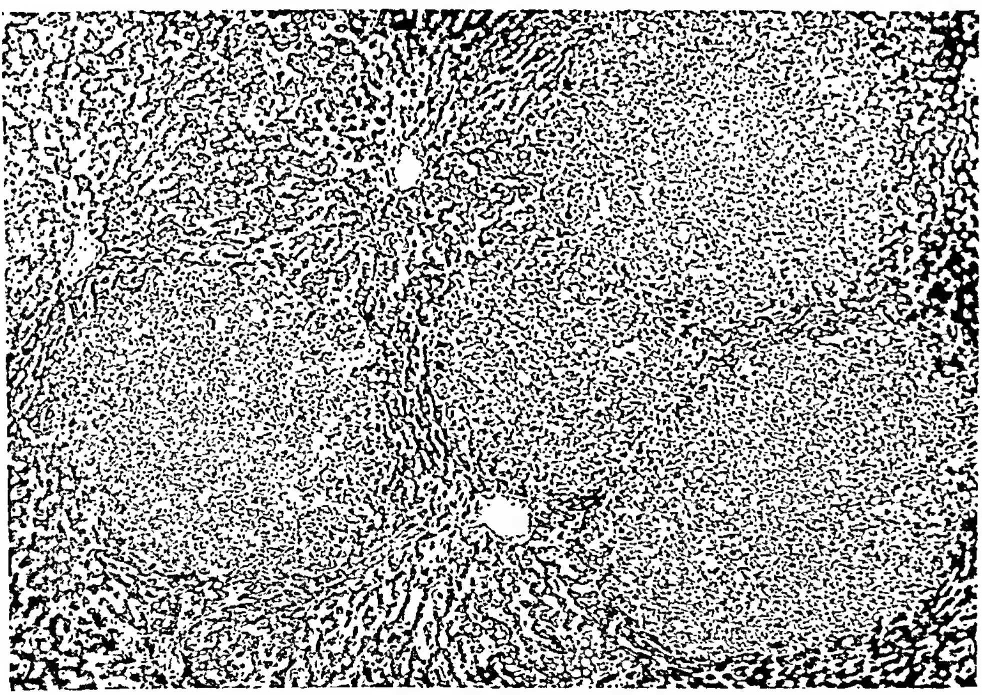


FIG. 12.—Focal necrosis of the liver in a case of lobar pneumonia in an infant male aged 9 months. The naked eye appearance suggested miliary tuberculosis ($\times$ *c.* 65).

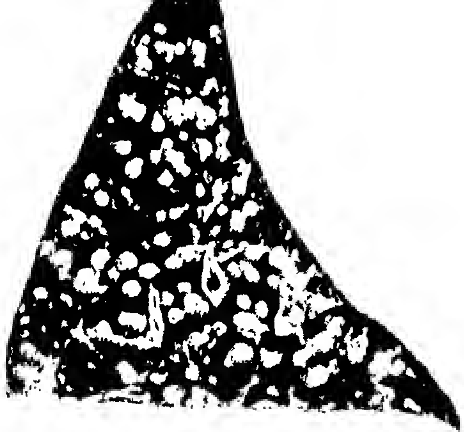


FIG. 13.

FIG. 13.—Cross section of a portion of spleen showing the greatly enlarged Malphigian bodies (3/4 nat. size).

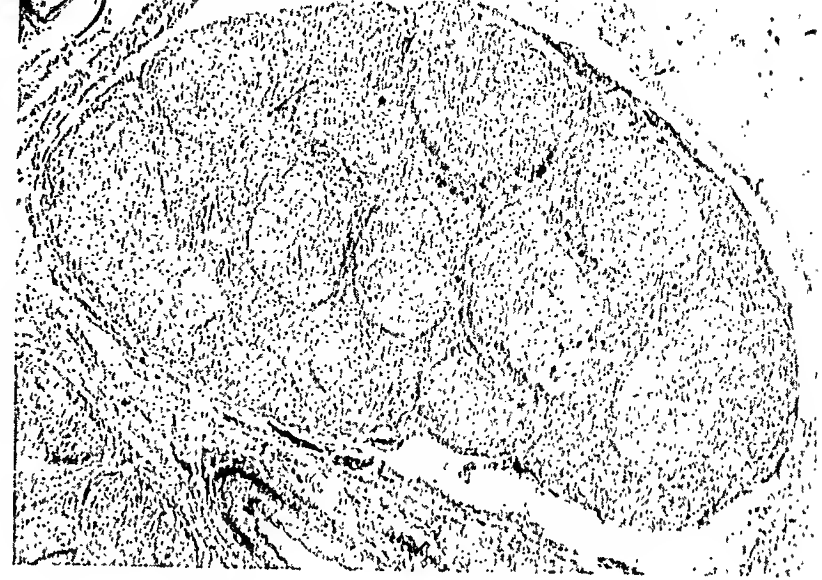


FIG. 14.

FIG. 14.—Cross section of a mesenteric lymph node showing the hypertrophied follicles (×18).

FIG. 15.—The caecum. Enlargement of the lymph follicles (3/4 nat. size).

FIG. 16.—Ileum. Hypertrophied Peyer's patches (3/4 nat. size).

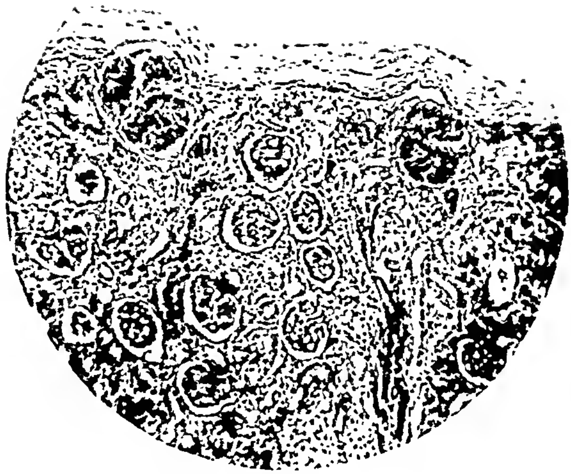
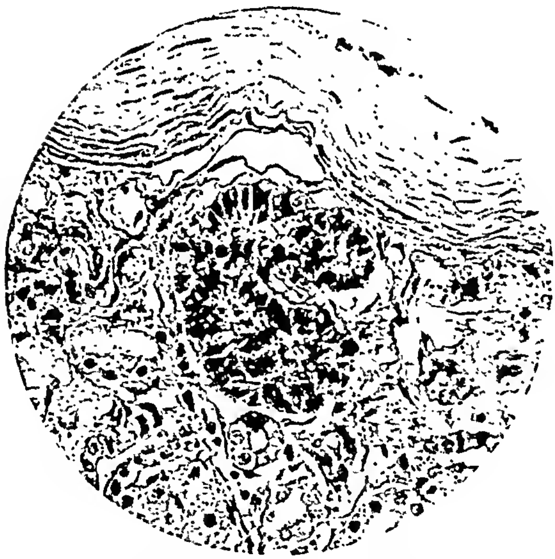


Fig. 17.



Figs. 17 and 18.—Kidney. Infant aged 1 month. Foetal stages of glomerular development. Colonel W. F. Harvey kindly reported on this section as follows : " Kidney with subcapsular glomeruli in considerable degree in a foetal state of development. The glomerular primordium is almost solidly cellular and although vessel formation can just be made out there is no real glomerular tuft nor is the glomerular tuft separated from Bowman's capsule. Even the well developed glomeruli show—as is diagnostic in infants —a two- or three-layered covering to the glomerulus. This specimen illustrates that organ development is not necessarily complete at birth."
(Fig. 17 × *c.* 70 ; Fig. 18 × *c.* 250).

Pulmonary Tuberculosis.

Thirty-two cases are recorded in this group. Eighteen were males and fourteen were females. The age groups are shown in Table IV.

The physical condition of the children in this group was very poor and in twenty-two cases the appearance was described as emaciated. The infection was, in general, of the acute type with cavitation following upon caseation. In two cases the necrosis was lobar in type (caseation pneumonia). Typical Ghon lesions were noted in six cases. Pleural adhesions (fibrous) were found on ten occasions, being unilateral in eight, bilateral in two.

Bronchiectatic cavities were seen once only.

That the infection had not remained localized to the thorax was demonstrated by the presence of tuberculous lesions elsewhere. In twenty-five cases lesions were present in the spleen, kidneys, liver and abdominal lymph nodes.

TABLE IV.

PULMONARY TUBERCULOSIS. AGE GROUPS.

Age groups.	Number of cases.
0–6 months	9
7–12 months	6
13 months–2 years	12
25 months–3 years	5

The youngest affected was aged 3 months.

In seven of these there was tuberculous ulceration of the Peyer's patches.

Tuberculous infiltration of the meninges was noted six times. In one of these cases a cerebral tuberculoma was present. Caseous cervical glands were recorded three times. The lesions were massive in one, an infant aged 4 months, suggesting that this was the primary focus of infection.

Bone involvement was noted in one instance ; a caseating lesion of the fourth rib.

A feature of the histology of many of the lesions was the absence of well defined tubercles or giant cells. It was possible, however, to demonstrate acid-fast bacilli in such lesions.

Diphtheria.

Diphtheria is rare in Nigeria, particularly so in Lagos. The infection now under consideration occurred in a well nourished male child aged 2 years. There was a history of fever for 2 days. The fauces, uvula, larynx and upper

part of the trachea were covered with a typical diphtheritic membrane. Stained smears revealed the presence of numerous Gram-positive bacilli, morphologically similar to *Corynebacterium diphtheriae*. The diagnosis was confirmed by the bacteriological findings which have been recorded by ELMES (1941).

MALARIA.

WHITBOURNE (1930) gives the estimated death rate from malaria as between 8 and 10 per cent. of the infant mortality. In the present series, seventy-two cases (14·4 per cent.) are recorded. There were thirty-seven females and thirty-five males. It is probable that malaria was a contributory factor in other cases since in 119 sections of liver (excluding the seventy-two cases of malaria) examined, well marked pigmentation of the swollen Kupffer cells was present. In a further forty-three sections of liver there was intense pigmentation of Glisson's capsule and of the Kupffer cells in the periphery of the lobules.

A feature of the malaria group was the well preserved and well nourished appearance of the children. Jaundice was noted in twenty cases. Of the seventy-two cases forty-seven were cerebral in type. It will be seen from the graph on p. 289 that the incidence was seasonal and in direct proportion to the rainfall. The age groups are shown in Table V.

TABLE V.

MALARIA. AGE GROUPS.

Age.	Number of cases.
0– 6 months	18
7–12 months	24
13 months–2 years	19
25 months–3 years	11

Further analysis of the first group showed that four cases occurred at 3 months, five at 4 months and nine at 6 months. All the cases of this group were cerebral in type. A presumptive diagnosis of malaria could usually be made on the naked eye findings, the more outstanding of which were briefly as follows.

(1) Enlarged and intensely congested spleen, slaty in colour and of a mushy consistency in cut surface.

(2) Intense pigmentation of the liver (usually enlarged). The cut surface frequently presented a mottled appearance owing to fat changes.

(3) Diffuse pigmentation of the lungs.

(4) Marked differentiation between the grey and white matter of the brain owing to the smoky discoloration of the former. Diffuse congestion and oedema were often present.

The diagnosis was finally established on the findings in the smears made from the spleen and brain.

FIELD'S stain (1940) was used during the latter part of the investigation and was found to be most satisfactory.

A diagnosis of cerebral malaria was made only when the capillaries in this organ were seen to be engorged with parasites. The recognition of parasites in the spleen was often difficult owing to their rapid degeneration, presence of diffuse pigment, laked red cells, etc. In most of the non-cerebral cases scanty parasites were found in the cerebral capillaries.

Changes in the Heart Muscle.

In eighteen of the seventy-two cases the heart muscle was flabby and pale and fat staining revealed well marked degenerative changes. No relationship could be demonstrated between the intensity of the infection and such changes. In several of the eighteen sections parasites were scanty whereas in sixteen instances in which no fat changes were present the parasites completely filled the capillaries of the heart.

Fat Changes in the Liver.

In twenty-eight of the seventy-two cases sections of the liver were negative for fat and though it was present to a varying degree in the remaining forty-four cases in only fourteen could the changes be described as extensive.

Haemoglobinuria.

MACKEY (1928) describes a case of blackwater fever in an African child aged 2½ years. Parasites were plentiful in the blood smears. In the present series haemoglobinuria was noted in two of the cases of cerebral malaria. Both were females and were aged 1 and 2 years respectively. In each case there was a history of fever and convulsions for several days. In both the urine was red-brown in colour and albumin was present (dense cloud). Microscopic examination revealed the presence of granular casts and the epithelial cells of various types but no red blood cells.

Spectroscopic examination confirmed the presence of haemoglobin in both.

In section both kidneys showed degenerative changes (fat stain) with epithelial desquamation and cast formation.

The diagnosis of cerebral malaria was made on the appearance of the smears but had the infection been less intense and more prolonged the changes in the kidneys might have constituted the deciding factor.

DISEASES OF THE ALIMENTARY TRACT.

This group consists of twenty cases and comprises :—

Colitis	9 cases	Intussusception (Ileo-caecal) ...	1 case
Cancrum oris	4 „	Typhoid	1 „
Amoebic dysentery ...	3 „	Ankylostomiasis	1 „
Gastritis	1 case		

Colitis.

The intensity of the colitis varied but diffuse inflammation and shallow ulceration were present in all of the cases. Necrosis of the ulcerated areas was noted in three.

Plating on MacConkey media was carried out in all with negative results except in one instance when an organism of the *B. lactis aerogenes* group was isolated.

Amoebic Dysentery.

Two liver abscesses were present in one case in which the colon showed numerous discrete ulcers with necrotic patches.

In a second case the ulcerations extended into the appendix.

Another case was associated with an acute generalized peritonitis as a result of multiple perforations of the sigmoid colon.

The diagnosis in all four cases of this group was made on the presence of well defined amoebae in section.

Typhoid.

The solitary case of typhoid occurred in a male child aged 2½ years. Generalized peritonitis was present and the ileum was found to be perforated about four inches from the ileo-caecal junction. The organism was isolated from the ulcerated Peyer's patches, the mesenteric glands and the spleen.

Appendix.

Although no death is recorded as a result of an inflammation of this organ it was found to be abnormal in eight cases. In six it was diffusely inflamed and in two it was bound to the posterior aspect of the caecum by numerous adhesions. Ascarides were present within the lumen of the appendix in four of the cases in which inflammation was noted.

Helminthic Infestation.

Helminths were present in 183 cases (36·6 per cent.). *Ascaris* was the most constant finding. "Hookworm" and *Trichuris trichiura* were frequently noted but the infestations were scanty and no counts were made except in two instances when 40 hookworms were noted in a child of 3 years and 200 in an infant aged

2 months. In the latter case there was diffuse inflammation of the gut combined with marked pallor of the viscera and ankylostomiasis was given as the cause of death. It is probable that the heavy infestation was due to the infant being placed on the ground close to a latrine. FISK (1939) mentions a similar case. This is the only case in the series in which death was attributed to a helminth infection and it would seem therefore that such infections do not constitute a vital factor in child mortality in Lagos. With the exception of the case just described helminths were not found in infants under the age of 6 months. The results of the *Ascaris* count are given in Table VI.

TABLE VI.

Ascaris COUNT.

Number of *Ascaris*	1	2	3	4	5	6	7	9	10	11	12	13	15	17	20	25	27	34	45	55	65	70
Number of cases	39	18	15	9	7	8	8	6	9	6	6	4	7	7	11	4	5	5	3	2	2	2

MENINGITIS.

Eighteen cases are recorded (excluding tuberculous meningitis) and pneumococci were isolated from fifteen. In seven the same type of pneumococcus was isolated from the meningeal exudate and from the lungs. Five were type 6 and one each types 2 and 14. In the remaining four cases the types of pneumococcus isolated were :—

Case	1	2	3	4
Lungs	T6	0	T6	T4
Meninges	T12	T6	T12	T23

These findings indicate that in three instances the condition was not secondary to a lung infection.

ANAEMIA.

In the absence of clinical histories and antemortem blood findings it was not possible to classify accurately these cases. Fourteen are recorded of which six were associated with intense jaundice, an enlarged, congested, finely

cirrhotic spleen and a marked degree of red cell sickling (fresh preparation of splenic pulp). In addition to these examples of acute sickle cell anaemia routine examination of fresh preparations revealed the presence of a latent sicklaemia in 24 cases (4·8 per cent.). Four cases were labelled splenic anaemia. The spleen in each was greatly enlarged, with well defined contours.

The cut surface was congested and dense and the Malpighian bodies were indistinct. Sickling was absent and no parasites or excess of pigment was noted.

The ages of the four children and the splenic weight are shown below.

Age	9 months.	12 months.	2 years.	3 years.
Weight of spleen in grammes	142	202·5	370	340
Normal weight. (FISCHER, 1922)	—	20·0	—	43

The remaining four cases were included under anaemia owing to the extreme pallor of the organs, positive Perles' test for iron pigment in the liver and absence of other findings which could be regarded as the cause of death.

MALNUTRITION (FAULTY DIET) AND/OR STARVATION.

It was not easy to determine the causal death factor in the fourteen cases included in this group but in four of them the naked eye and histological findings were considered to be indicative of a form of avitaminosis. They are therefore described in some detail.

The children were males, aged 5 months, 10 months, 1 year and 2 years respectively and all were emaciated.

The lips were excoriated and the oral angles showed the whitish dry patches typical of this condition. The scrotal skin was atrophic, dry, shiny and finely wrinkled with epithelial desquamation in two of the cases. In one there was some superficial ulceration of the skin of the buttock.

The outstanding feature at autopsy was the appearance of the liver. This organ was enlarged, ochre-yellow in colour and of a soft almost doughy consistency. The cut surface was haemogeneous, deep buff yellow in colour with absence of the normal lobulations.

In the two cases in which the bladder was distended the urine contained albumin (dense cloud on boiling and addition of acetic acid). A terminal bronchopneumonia was present in all of the cases.

Histology. (Scarlet red stain.)

The liver presented a remarkable degree of fatty change. The hepatic cells were completely replaced by large fat globules and no normal tissue was discernible with the exception of the portal tracts.

Fat changes were also present in the convoluted tubules of the kidneys.

WRIGHT (1928) described a form of avitaminosis in Sierra Leone which was associated with sore tongue, perlèche and visual disturbances.

WILLIAMS (1935) has described a similar condition in the Gold Coast and TROWELL (1940) in Uganda. MOORE (1937) has drawn attention to the occurrence of retrobulbar neuritis in connection with avitaminosis in Nigeria.

CLARK (1936) showed that cassava foodstuffs retain cyanogenetic glucosides even after they have been prepared for food and he put forward the theory that the cyanogens, by combining with the sulphur of the proteins deprived the body of this vital constituent. If we assume that the factor concerned in avitaminosis is contained within the sulphur content of the proteins the harmful results which might follow the prolonged ingestion of such foodstuffs is obvious. CLARK maintains that fatty degeneration, particularly of the liver and kidneys can be produced by a diet in which cassava predominates and that the prolonged ingestion of cassava gives rise to albuminuria. Coco yam, on account of its glucoside (saponin) content, produces the same result. It follows that, other factors being excluded, the incidence of albuminuria should be more common in Southern Nigeria than in the Northern territories where coco yam and cassava are not consumed in any quantity. The incidence of fatty degeneration of the liver and kidney should also be higher. An analysis of the incidence of albuminuria and of fat changes in the liver and kidneys for the 500 cases under consideration follows.

ALBUMINURIA.

In 292 cases the bladder was retracted and no urine was obtainable. A specimen of urine in the remaining 208 cases was submitted to the heat and acetic acid test with the results stated :—

Albumin negative ...	...	28 cases	...	13·46 per cent.
„ faint cloud	...	94 „	...	45·18 „
„ dense cloud	...	86 „	...	41·46 „

Of the 208 cases, therefore, albuminuria was present in 180 or 86 per cent.

Kidneys.—Fat changes.—Frozen sections were prepared from 350 specimens and were stained by scarlet R. In 112 or 32 per cent. well defined fat changes were present in the parenchymatous cells of the convoluted tubules.

Liver.—Fat changes.—A specimen of liver was examined in every case for the presence of fat and the findings are given in Table VII.

Had the number of cases in the disease groups been more uniform an aetiological analysis of the positive fat findings would have been of interest. It is noteworthy that, apart from the cases of avitaminosis, fat changes were markedly consistent in the liver from the cases of tuberculosis. Of the thirty-two recorded cases only two were negative and in the remaining thirty the changes included a total replacement of the liver parenchyma in eleven cases, P+++ in fourteen and P+ to P++ in five.

TABLE VII.

LIVER. FAT CHANGES IN.

Degree of fat changes	Number of specimens.	Percentage.
Negative	210	42
P+	56	11·2
P++	37	7·4
P+++	22	4·4
++++	49	9·8
C+	59	11·8
C++	35	7·0
P+C	9	1·8
Diffuse patches	23	4·6
Total positive	290	58

Key.

P+ to P+++ = Varying degree of peripheral fat distribution.
P+C = Changes both peripheral and central with midzonal free area.
++++ = Hepatic cells completely replaced by fat globules.
Diffuse patches = Scattered fatty areas following no zonal distribution.

These figures, namely, the albuminuria rate of 86 per cent., a positive fat percentage of 58 per cent. in the livers and 32 per cent. in the kidneys tend to support CLARK'S thesis but in the absence of comparative figures from other parts of West Africa it would be unwise to advance any conclusions.

CONGENITAL GROUP.

The eight deaths in this group comprise :—

Icterus neonatorum	3 cases, one with complete stenosis of the common bile duct and two of the cystic duct.
Cystic kidney	1 case.
Hydrocephalus	1 ,,
Patent foramen ovale	1 ,,
Bilateral suprarenal haematoma ...	1 ,,
Syphilis	1 ,,

The last three cases merit further comment.

(1) The first (patent foramen ovale) was an infant 8 days old. No history was available.

There was little of note to be seen at the autopsy apart from the large interatrial septum. In addition to this case a patent foramen ovale was noted

fifty-five times (11 per cent.). In most the opening was small, varying from 2 to 5 millimetres in diameter but in one instance, a male child aged 8 months, the opening measured 2 cm. in its longest diameter.

The cause of death in this case was a lobar pneumonia. The ages of these cases with patent foramina varied. Thirty-three were under 6 months, fifteen under 12 months and seven were between 1½ and 2 years of age. A small opening in the interventricular septum was found in a male child aged 2½ years.

(2) The congenital syphilitic was a male infant, aged 4 weeks. It was stated that he had had convulsions for 4 days. His general condition was poor and jaundice was intense. The liver was pale brown in colour and weighed 200 grammes. There was nothing of note to be seen in the other organs with the exception of the heart which was pale and flabby, and the lungs which were mildly bronchopneumonic with some fibrosis. The histological appearance of the liver was identical with that depicted by MacCallum (1937) and large numbers of spirochaetes were demonstrated by the Warthin method.

The lungs showed no excessive fibrosis.

Both the kidneys and the heart were positive for fat.

(3) The bilateral suprarenal haematoma occurred in a male infant aged 2 weeks. The left suprarenal ruptured during removal and exuded a dark brown fluid. The kidneys appeared to be normal.

MISCELLANEOUS.

Under this heading are included :—

Birth injuries	1 case.
Biliary cirrhosis	2 cases.
Myocarditis	1 case.
Multiple liver abscess	1 ,,
Peritonitis (pneumococcal, T 21 Lungs T 6) ...	1 ,,
Toxaemia (associated with retropharyngeal abscess)	1 ,,
Unknown	4 cases.

Biliary Cirrhosis.

One of the two cases was a female child aged 5 months, the other was a male aged 3 years. In both the general condition was excellent. Jaundice was present. The livers were enlarged, intense green in colour and with a morocco leather surface. Gross section showed a fine cirrhosis.

In the first case the cystic duct was stenosed but allowed the passage of a fine probe. The gall bladder was atrophic and contained inspissated mucus.

In the second case numerous adhesions were present straddling the hilum of the liver and involving the common bile duct. The gall bladder was distended with thickened bile which could be made to exude at the ampulla of Vater only by the application of considerable force. The histological findings in both cases

were characteristic of an obstructive biliary cirrhosis. It might perhaps have been more correct to have included these cases with those of congenital origin but the presence of adhesions in the second suggested the possibility of a pre-existent peritonitis. The lungs in both were bronchopneumonic.

Myocarditis.

This case was a male aged 1 year. The general condition was good. The left auricle was dilated and the left ventricle was elongated and presented a fibrosed aneurysm-like swelling at the apex. The inner surface of this fibrous dilatation was covered with an antemortem clot.

The adjacent muscle tissue was streaked with white and the papillary muscles were typically thrush-breast in appearance.

The valves were normal and no obstruction could be found in the coronary arteries. Of the remaining organs the kidneys were swollen and pale (albuminuria, dense cloud) and the lungs showed a well marked haemorrhagic type of bronchopneumonia.

Although this case is the only one recorded in which the condition of the heart is cited as being the primary cause of death there were many instances in which the heart muscle was noted as abnormal. Routine sections, stained for fat, were positive in seventy-six specimens (15·2 per cent.). This figure includes the eighteen positive findings alluded to under malaria.

SUMMARY.

An analysis of 500 postmortems on children up to the age of 3 years is given ; it consists of general statistics which are elaborated under the various disease groups : under the respiratory group, which totals 343 cases, the various forms of pneumonia are detailed together with a table of the types of pneumococci isolated. Thirty-two cases of pulmonary tuberculosis receive comment and a case of diphtheria is described. There were seventy-two cases of malaria, forty-seven of which were cerebral in type. The naked-eye appearance of the organs ; the pigmentation of the liver, together with fat changes in the latter and in the heart muscle are described and mention is made of the two cases which were associated with haemoglobinuria. Two cases of amoebic dysentery, one of typhoid, and the helminthic findings are included under diseases of the alimentary tract (twenty cases). Meningitis (excluding that brought about by the tubercle bacillus) accounted for eighteen deaths. The classification of the anaemias was difficult owing to the absence of antemortem findings but six of the fourteen cases in this group were sickle cell in type and four were regarded as splenic in origin.

Fourteen cases were included under malnutrition and the findings in four of these were considered typical of avitaminosis. The salient naked-eye and histological features are described and reference is made to the work of various

investigators of this condition in West Africa. The incidence of albuminuria was found to be approximately 86 per cent., a surprisingly high figure. Fatty changes in the liver are tabulated and the total positive findings amounted to 58 per cent.

The congenital group (eight deaths) includes several cases of interest. Patent foramen ovale is given as the cause of death in one and a note on the incidence of this condition follows. A case of bilateral haematoma of the supra-renal is noted. The fact that only one case of syphilis is recorded in this group indicates the unimportance of this disease in Lagos as a factor in child mortality.

Under the miscellaneous group (eleven deaths) are included two cases of biliary cirrhosis and one of myocarditis. A routine histological examination of the heart muscle revealed degenerative changes in 15·2 per cent.

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THE BACTERICIDAL EFFECT OF TIN AND ITS APPLICATION TO THE TREATMENT OF TYPHOID FEVER.

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I.—THE BACTERICIDAL EFFECT OF METALLIC TIN ON BACTERIAL SUSPENSIONS.

From the comprehensive study made by HOTCHKISS (1923) on the bactericidal effect of metal ions it can be seen that Sn occupies approximately an intermediate position between the most bactericidal Hg and the two least efficient metals Na and K, the lowest growth inhibiting molar concentration of $HgCl_2$ being 0·00001, of $SnCl_4$ 0·005, and of NaCl and KCl 2·0. This fact seemed to be interesting since Sn, while still exhibiting a considerable bactericidal power, is less toxic for man than the heavier metals.

The investigations of HOTCHKISS were carried out by the use of chlorides of various metals against *Bacillus coli.* In testing one metal against various micro-organisms we thought it more suitable to use the oligo dynamic effect of the elementary metal, as many water-soluble salts—tin salts included—yield acid solutions due to hydrolysis, and the various bacteria differ widely in their susceptibility to H-ions (WINSLOW and LOCHRIDGE, 1906). Also, in view of the ultimate use of metallic tin or insoluble tin salts for therapeutic purposes, this method should be nearer to the conditions *in vivo.* On the other hand, such experiments supply us only with comparative data concerning the different susceptibility of various micro-organisms to the metal tested, without yielding well-defined figures of concentrations. Our investigations on these lines revealed that metallic tin has an elective bactericidal effect on certain bacilli only, while leaving others entirely unimpaired.

The experiments have been arranged in the following way:

1. Thirteen tubes, each one containing 1·0 gramme of spongy powdered metallic tin and 10 c.c. normal saline solution, are sterilized by steam, and the tin is allowed to settle down for two days. After this time the supernatant fluid is poured off, and replaced by 5·0 c.c. of sterile Ringer's solution.

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The tubes are thoroughly shaken so that the whole tin is suspended and then kept for two days at room temperature, till the metal has settled down again. At the same time, thirteen more tubes are prepared with 5·0 c.c. of Ringer's solution without tin for controls.

2. One day after the preparation of the tin in Ringer's solution slope agar cultures are made from the bacterial strains :—

TABLE I.

Name.	Source.
Eberthia typhi H_{901} ...	Laboratory strain
Escherichia coli	Human faeces
Salmonella paratyphi A ...	Laboratory strain
" " *B* ...	" "
" *enteritides* Gaertner	" "
Bacillus dysenteriae Shiga	Human faeces
" " Flexner	" "
B. proteus	Dead rat
Pseudomonas pyocyanea	"
Pasteurella pestis	Human bubo
Brucella melitensis ...	Laboratory strain
Staphylococcus aureus ...	Human boil
Enterococcus	" faeces

After 24 hours' incubation the growth is washed off with normal saline solution and the suspensions are standardized to 1,000 million of organisms per c.c.

3. One tube with tin and one control tube without it are now inoculated with 0·05 c.c. of the same bacterial suspension, taking care that the tin at the bottom is not stirred up to avoid any mechanical absorption. Thus, the Ringer's solution contains now 10 million organisms per c.c. in each tube. The inoculated tubes are kept at 37° C.

4. After 1- 2- 3- etc., up to 10 days' incubation

(*a*) One loopful of each tube is plated daily on an agar plate, and the number of colonies is counted after 24 hours' incubation. As the loop contained 0·0025 c.c. the number of organisms per loop was 25,000 at the beginning of the experiment.

(*b*) 0·1 c.c. of each tube (= 1 million organisms at the beginning of the experiment) is put daily into 10 c c. of broth and the presence or absence of growth after 24 hours' incubation is recorded.

A decrease of colonies below 100 in test (*a*) indicates a considerable reduction in number of the organisms ; absence of growth in tests (*a*) and (*b*) is practically tantamount to a sterilization of the fluid.

The results of these experiments may be summarized as follows :

1. No effect of tin was observed on *Enterococci, Salmonella enteritides* Gaertner, *Pseudomonas pyocyanea*, *B. proteus* and *B. dysenteriae* Shiga.

2. All the other organisms tested were gradually reduced in number as compared with the controls and eventually destroyed completely after various periods of contact with the metal, as shown in Table II.

TABLE II.

Organism.	Number of days necessary for	
	Reduction of colonies below 100.	Sterilisation.
P. pestis	1	2
E. typhi	4	6
S. paratyphi A	4	6
S. aureus	4	6
B. dysent. Flexner ...	5	5
E. coli	5	7
B. melitensis	7	10
S. paratyphi B	7	9

As soon as a considerable reduction in the number of colonies became obvious the bacterial suspension with tin became markedly clearer in comparison with the control which retained its initial slight turbidity. After sterilization took place the fluid was completely clarified.

All the strains which were eventually killed by tin with the exception of *E. typhi* and *S. paratyphosus B.* grew in dwarf colonies in the last plate cultures before they were destroyed. These colonies contained numerous involution forms and, in the case of *B. dysenteriae* Flexner, their agglutinability was completely lost.

Repeated experiments on the same lines but partly with other strains of *P. pestis*, *E. typhi* and *S. aureus* gave in principle the same results, although the time necessary for destroying the same kind of bacilli varied somewhat from one experiment to the other.

The gradual reduction in number of colonies spread over several days indicates that, as with other disinfectants, the resistance against tin of the individual organisms in the same culture varies considerably.

II.—THE THERAPEUTIC USE OF TIN IN TYPHOID FEVER.

In view of the two facts, namely, that typhoid bacilli proved to be to the same degree susceptible to the bactericidal action of tin as *Staphylococci*, and that tin preparations have been successfully used against staphylococcal infections of the skin (FROUIN, 1917 ; LAWRENCE, 1922 ; CRHA and JINDRICH, 1922,

and many others) and the bone marrow (KLEIN, 1932), the therapeutic use of tin against typhoid fever could be envisaged. But before reporting on our clinical experiences, we shall briefly review the toxic effect of tin and its fate in the mammalian body, as this knowledge will indicate in advance the probable limitations of the therapeutic effect of tin.

Water-soluble inorganic tin salts are caustic (ORFILA, 1818) and organic salts, while not causing local irritation, are more or less toxic (WHITE, 1881; UNGAR and BODLÄNDER, 1887), the main effects being initial irritation of the central nervous system, followed by paralysis and intestinal colic. The kidneys, however, remain unimpaired. The toxic effects are more pronounced if the salts are injected, but may be brought about by oral administration as well. A given total amount is more toxic if administered over a few days than the same or a higher one spread over a longer period. Insoluble tin compounds are supposed to be absolutely harmless and may become toxic only if they undergo a change in composition in the body. But so far no reports in this respect have been forthcoming.

Tin salts are found in the blood only a short time after having been injected (WHITE, loc. cit.; UNGAR and BODLÄNDER, loc. cit.), and may disappear after a few hours (SALANT, RIEGER and TREUTHÄRDT, 1914). It is, therefore, impossible to maintain an appreciable tin level in the blood for any prolonged period. The metal is rapidly deposited in the tissues, particularly in the lymphatic organs of the intestines (solitary follicles, Peyer's plaques, mesenteric lymph glands) not only after oral, but also after parenteral administration (UNGAR and BODLÄNDER, loc. cit.), and, furthermore, in the bones, skin, muscles and liver; in small amounts it is found in the kidneys, spleen, brain, heart and stomach wall (SALANT, RIEGER and TREUTHARDT, 1918).

The metal is mainly eliminated by the faeces and to a lesser degree by the urine (SALANT *et al.*, loc. cit.). Even if large doses of soluble salts are given daily by mouth to experimental animals, elimination by the kidneys starts only after one week's feeding (BUCHANAN and SCHRYVER, 1908). This is probably due to the process of depositing the metal in the body. But once it has been stored, the tissues retain it for a long time and elimination goes on for up to one month after discontinuing the administration (HANDOVSKY, 1926). No experiments have been carried out, as far as we could ascertain, concerning resorption, storing and elimination of colloidal tin or insoluble tin salts, which may be split up in the intestines after oral administration. But in view of the findings of UNGAR and BODLÄNDER we may safely assume that these substances, if resorbed at all, will be predominantly deposited and retained for a considerable time in the lymphatics of the intestines.

We cannot, therefore, expect a direct influence on typhoid bacilli in the blood by administration of tin, the less so in that we have to bear in mind that oligodynamic effects are even more hampered by a colloidal medium than other disinfecting actions. Short contacts between metal and bacilli in such a medium

will be of no avail. Yet, there is *one* common site of lasting accumulation of metal and typhoid bacilli, namely, the intestinal lymphatics. If the duration of contact here is long enough (and that may easily be the case, in view of the long-lasting retention of tin) we may expect a certain sterilizing effect at this site and perhaps to a lesser degree in the liver and bone marrow. All the other tissues are either not particular concentration points of the typhoid bacilli or do not accumulate tin in any appreciable amounts. It remained to be seen whether such locally limited effects would suffice to influence favourably the course of the disease, if not in all, then at least in the majority of cases. This question could be decided only by clinical trials. At any rate, if such an influence exists at all, it could hardly manifest itself in a very dramatic way, as a certain degree of saturation of the tissues with tin should be a prerequisite to the bacteriocidal action, and this point could probably not be reached very quickly.

We used for our clinical trials a commercial product in tablets for oral use containing a mixture of colloidal metallic tin and tin stearate with a total amount of 0·012 gramme tin per tablet.

The drug is absolutely devoid of any toxic action in healthy persons, even if taken in excessive doses. The resorption of tin after oral administration to man was demonstrated by the presence of tin in the urine after ten tablets daily were taken for 10 days. No attempts have been made so far to investigate the chemical changes the drug may undergo in the intestinal tract, nor to follow up the elimination in a quantitative way.

Dogs of 5 kg. body weight were fed for a period of 15 days with five tablets and for 30 days with two tablets daily. All the animals were still in perfect health when they were sacrificed after these periods. The postmortem examination revealed a considerable enlargement of Peyer's plaques and of the mesenteric lymph nodes, as well as a hypertrophy of the intestinal mucous membrane*.

The optimal doses we were able to ascertain in a series of preliminary trials in typhoid cases as given in Table III.

TABLE III.

Age in years.	Number of tablets per day (taken in 2 to 4 equal doses).	
	1st day.	Following 9 days.
Up to 2	4	2
2 to 5	8	4
6 to 11	12	6
12 to 15	16	8
Above 15	20	10

* We are indebted to the producers of the drug, Dr. R. MARBERGER and Dr. F. ZIPSER for supplying us with these experimental data.

The tablets are given for a period of 10 days in doses according to the age of the patient, the dose on the first day being double the amount on following days.

In these amounts no serious toxic effects whatsoever could be observed. Lower doses rarely caused a decrease in temperature, as will be described later, and higher ones had a definite depressive effect on the leucocytes.

Yet, it was noteworthy that in most of the cases treated with the doses indicated above, a slight rise of temperature could be observed after the first or second day of treatment, and complete defervescence took place only after discontinuing the treatment ; in several cases slight subfebrile evening rises of temperature could be recorded for many days more. First, we tried to overcome this latter inconvenience by shortening the duration of treatment while increasing the daily doses. But this led to the impairment of leucocytes as seen in the following case :

Case 1.

Ahmed H., male, 18 years old. Admitted to hospital with high fever and intestinal haemorrhage on the 22nd day of disease. Blood culture : *B. typhosus* +. After the haemorrhage had been stopped, he was given on the 28th, 29th and 30th day (in four doses) ten tablets daily and the same doses on the 37th, 38th and 39th day. Temperature started to come down on the 41st day and reached the normal level on the 46th day. Ten days later the patient was discharged. Blood counts were made on the 28th, 33rd, 37th, 41st and 52nd day (Table IV).

Table IV.

LEUCOPENIA DUE TO OVERDOSAGE OF TIN IN A TYPHOID CASE.

	Day of disease.				
	28th.	33rd.	37th.	41st.	52nd.
Total number of leucocytes ...	*4,600*	*2,000*	*2,600*	*1,800*	*5,300*
Neutrophiles—band forms ...	10	15	15	16	6
segmented forms . .	19	23	26	22	41
Lymphocytes	70	60	58	61	50
Mononuclears	1	2	1	1	3
Eosinophiles	0	0	0	0	0

We see a reduction of leucocytes by half, 3 days after the last dose, and a slight recovery after another 4 days. The next administration of tin is again followed by a considerable drop in the leucocyte number which, however, turns into a nearly normal figure 11 days later, that is to say, 13 days after the last administration of tin. The lymphocytes are somewhat more involved in this reduction than the neutrophiles. The red blood picture never showed any changes which might be attributed to the effect of tin.

The decrease in leucocytes was always accompanied by a certain mental dullness and apathy which improved or got worse in accordance with the white blood picture. It was particularly obvious in this case as before treatment the patient did not show any mental symptoms of toxaemia. Both these effects

were nearly the contrary of what we observed with moderate doses, as will be seen later. Yet the patient was given only slightly more of the drug in each course than the patients treated with smaller doses. Only the whole amount, otherwise spread over 10 days, was given here in 3 days (cf. what is said above on the relation of toxic effects and duration of administration).

The slight mental symptoms of this patient together with the known nervous effects of tin poisoning, led us to the idea that even in moderate doses the drug has a stimulating effect on the thermo-regulatory centre—already rendered more sensitive by the infection—and, that this stimulation should be amenable to the action of antipyretics. This conjecture proved to be correct. In all cases where the tin treatment may have had any shortening effect at all on the course of the disease the temperature was brought to normal within a few days in this way, and remained so after discontinuing the use of antipyretics. In cases where the course was not shortened, the antipyretics had only a very transient effect. Therefore, we administered, as a rule, small doses of acetyl-salicylic acid (0·15–0·3 gramme) or 0·5 gramme sod. salicyl. three times daily for adults, and reduced doses for children, from the 8th day of the tin treatment onward for about a week. If the temperature did not become normal by that time, the use of antipyretics was discontinued and another course of tin was started one week after termination of the first one.

No incompatibilities have been observed with other drugs (heart stimulants, sedatives) with the exception of sulphapyridin in some cases suffering from concomitant pneumonia, where both drugs together caused severe vomiting. Later on, we found that the combination of tin with *injections* of sulphapyridin was well tolerated. This fact is important as pneumonia is sometimes a frequent complication of typhoid.

As mild cases would give inconclusive results anyway, we decided to exclude them altogether from treatment and to carry out our experiments on severe ones only. Amongst them even the most hopeless-looking patients were treated, and only those left out who eventually died within the first 24 hours after admission. Since fatalities occur exclusively amongst the severe cases, the death rate in such a selected group should be considerably higher than the total death rate from typhoid in the same place during a previous period of equal average severity of the disease, as this latter figure would be calculated on the basis of all cases, including the mild ones. A lower death rate in the treated group would be a definite proof of a favourable effect of the treatment.

This plan appeared to be the most suitable one for gaining an opinion on the value of a drug which was not supposed to yield quick results. Accordingly, we have treated 100 typhoid cases admitted to hospital in an obviously serious condition between 10th November, 1941, and 15th June, 1942, and compared the death rate of this group which comprised thirty-four highly and thirty moderately toxic cases with the death rate of all typhoid cases admitted to our hospital between 1st November, 1940, and 31st October, 1941. There was no difference

in the general character of the disease during these two periods and the various age classes were equally represented in both groups. Deaths occurring within the first day of hospitalization have also been eliminated from the control group.

Fifty-five out of the 100 treated patients had positive blood cultures, and 117 (27·3 per cent.) out of 428 controls likewise. In the other cases, diagnosis was established either by agglutination or by culture from stools or urine.

The result of this comparison was most striking: we had only 3 fatalities among the 100 severe cases treated with tin, as compared with 35 (8·2 per cent.) deaths in the 428 severe and mild controls. The mortality was, therefore, roughly two and a half times higher in the untreated group, in spite of the fact that it included about 30 per cent. of clinically mild cases. Thus, this crucial experiment decided clearly in favour of the tin treatment.

A less spectacular effect was obtained with regard to the temperature, and that could hardly be otherwise in view of the supposed mechanism of action of the drug. The fever never dropped by crisis. Following the initial slight rise, a gradual lysis commenced usually between the 5th and 10th day of treatment in 58 cases and mostly with the help of antipyretics temperature became permanently normal in these cases about 6 to 15 days after the first administration of the drug (Chart 1*).

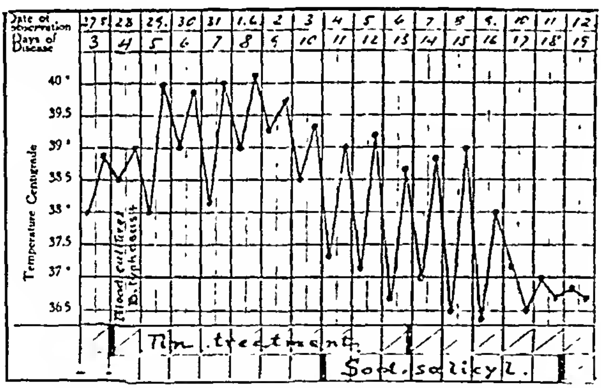


CHART 1.
Case of typhoid fever reacting favourably to tin treatment.

A.G., male, 25 years. Admitted 27-5-42. Discharged 20-6-42. Recovery.

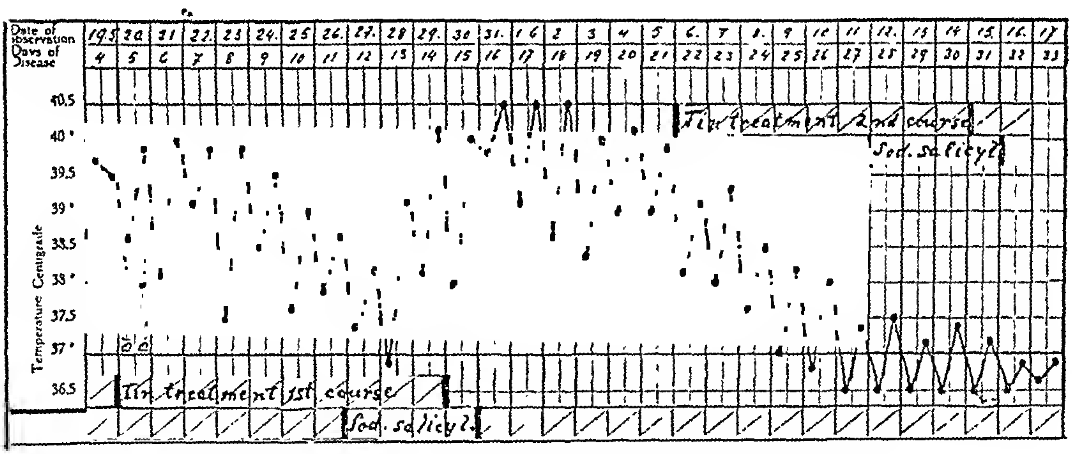
If we take into consideration that treatment was started at the earliest towards the end of the first week of disease, and in many patients later, we should say that each of these cases considered singly could have taken such a course equally well without treatment. But it cannot be a mere coincidence that this course was the same in so many severe cases. We considered, therefore, all these cases as favourably reacting so far as defervescence was concerned, being

* Our fever charts are maximum minimum curves, that is to say, they do not show the temperature at 6 a.m. and 6 p.m., but the highest and lowest temperature of the day entered as they occurred either before or after noon.

satisfied that no quicker effect could possibly be expected and that the length of the illness had been most probably shortened in these patients. The average duration of fever counted from the first day of treatment was in this group 10·3 days with a shortest duration of 4 and a longest one of 15 days.

A second group of twelve cases was constituted by those patients who reacted perfectly favourably on the first course, but had one relapse after a varying interval of apyrexia. The average duration, including the apyretic interval and the relapse amounted here to 26 days, the minimum being 14 and the maximum 48 days. It should be pointed out that these relapses responded as a rule much more quickly to repeated treatment with tin than the first attack to the first course of treatment. The question arises whether some of them could not have been avoided if we had given, as a routine, a second course to every patient irrespective of the more or less satisfactory result of the first.

A third group comprising thirteen cases was particularly interesting. The fall of fever started in these patients in the same way as in the first group, namely, lytically towards the end of the first week of treatment, but during its last days temperature rose again in spite of the administration of antipyretics and continued from now on as in an untreated case (Chart 2). This similarity



Z.G., female, 26 years. Admitted 19-5-42. Discharged 24-6-42. Recovery.

CHART 2.—Case of typhoid fever incompletely reacting to tin treatment.

in the general trend of the fever in the beginning seems to indicate an initial though eventually insufficient effect of treatment on temperature, and might be interpreted as due to an incomplete destruction of the bacilli in the beginning followed by a multiplication of the more resisting individuals. During or after the second course of tin the temperature usually came down, but it is impossible to state whether this can be still attributed to the treatment. We classified these cases as incompletely reacting. Their average duration of fever after commencement of treatment was 18 days (minimum 15, maximum 26 days).

A fourth group of seventeen cases with an average of 28 fever da
19, maximum 42 days) did not show any reaction as far as tem
concerned, in spite of the administration of antipyretics which
fever for a few hours only. Three patients of this group had c
relapses.

The fact that in the three last groups the effect on the feve
incomplete or lacking altogether does not, of course, mean that t
had no effect whatsoever in these cases. They certainly would ha
share in deaths if they had not been treated, and many of them ma
lasted longer but it is still more impossible to say anything definit
latter point in these three groups than in the first one.

A surprising observation was made when the treated cases we
according to the period of disease when treatment was started in re
course of the temperature. The percentage of favourable effects c
was somewhat higher amongst the cases whose treatment was starte
first 10 days of disease than amongst those started later (Table V).

TABLE V

RELATION BETWEEN PERIOD OF ILLNESS WHEN TREATMENT WAS STARTED AND COUR

Commencement of treatment.	Effect.			
	Favourable.	Favourable with relapses.	Incomplete.	None.
Within the first 10 days	43 (60·6 per cent.)	8 (11·3 per cent.)	9 (12·6 per cent.)	11 (15·5 per cent.)
After	15 (51·7 per cent.)	4 (13·8 per cent.)	4 (13·8 per cent.)	6 (20·7 per cent.)
Total	58	12	13	17

If the drug had no effect on the temperature the contrary should
found. The patients who started treatment in the later stages would
nearer to the natural end of the fever and this should more frequent
with the treatment than in the earlier started cases, who would still
them the whole length of the disease. This finding confirms our o
the treatment shortens the duration of the illness. From a practic
view we concluded that it was advisable to start treatment at onc
suspicious case, without waiting for a laboratory confirmation, espec
drug was harmless in all cases where we did so and where the
eventually did not turn out to be typhoid.

The comparison with regard to the duration of the illness by tr

alternate cases of apparently equal severity is very difficult in endemic typhoid where we have to deal with patients from various social strata widely differing in resistance and coming from different localities where the infection may be due to strains of different virulence. Such a comparison is feasible only in local epidemics affecting more or less the same type of population, or, still better, with children from the same family and of the same age group falling ill at the same time. We had the opportunity of observing one couple of this kind and the result was rather convincing :

A weakly boy, 9 years old, and his much better developed sister of 12, fell ill on the same day. Blood culture was in both cases positive for *B. typhosus*. The boy's condition soon became very serious and toxic with hyperpyrexia (40 to 41°C.) tachycardia (pulse rate 104 to 128) and slight cyanosis, and all signs pointed to a severe and long illness or a fatal outcome. The girl also ran a high temperature (40 to 40·5° C.), but had the usual relative bradycardia (pulse rate 86 to 100) and no particular signs of toxaemia during the first week. The boy was treated with tin from the 7th day of disease till the 16th, and a second course was given between the 25th and 34th day. From the 12th till the 16th day he suffered from bronchopneumonia which was treated with soludagenan. In spite of all that temperature became permanently normal on the 26th day and further recovery was uneventful. The girl was left untreated and later on became slightly toxaemic although not as severely as her brother. Between the 23rd and the 28th day she suffered from pneumonia as well, was treated with soludagenan and reached permanently normal temperature only on the 38th day. Although the boy could be classified only as incompletely reacting with regard to the apparent effect on the fever, the duration was nevertheless considerably shortened as compared with the duration of fever in his untreated sister.

The effect of treatment on toxaemic symptoms (tachycardia, delirium, unconsciousness) varied considerably. In many toxic cases an appreciable improvement in this regard took place much earlier than could have been expected, namely, about 3 to 6 days after commencement of treatment. But if by this time toxaemia had not begun to subside, its further course was hardly different from the one we were accustomed to in non-treated cases. In some toxic cases the usual mental dullness became a very prominent feature during treatment, so much so that we were even inclined to attribute it partly to the effect of the drug, bearing in mind our experience with overdosage. But yet it subsided often before treatment was terminated while in other cases other toxic signs as, for instance, tachycardia, decreased markedly while the mental symptoms were still progressing, so that we were finally unable to decide to what an extent, if at all, the drug should be held responsible for it. At any rate, it was a perfectly harmless and transient symptom which should never justify cutting short the *first* course or reducing the doses. Only in one case of extremely severe toxaemia with acute delirium, the mental symptoms began to subside gradually after the termination of the first course, but as soon as a second one was started they got worse and heart action became irregular so that we discontinued treatment after three days. The patient eventually recovered and this remained the only case where an untoward effect definitely followed the doses indicated above.

We prefer to withhold, for the time being, our opinion on the effect of the

treatment on complications. They occur always in a small fraction of cases only, and our total number of observations has to be much higher in order to permit safe conclusions. But a few words have still to be said about the effect of the drug on the leucocyte picture. In twelve cases we followed up the blood picture by counting on the day when treatment started and when it ended, and in three cases also in the middle. The result may be summarized as follows: the red blood picture deteriorated neither more nor less than we usually see in untreated typhoids. The leucocytes, on the other hand, decreased considerably less in number than we would expect, and, in four instances, there was even a slight rise in the lymphocytes as, for instance, in Case 2:

Case 2.

Jamileh R., female, 25 years old. Admitted on the 30th day of disease with high fever in a very emaciated and toxaemic condition. Blood culture *B. typhosus* +. Tin treatment from 31st to the 40th day of disease; blood counts on the same two days (Table VI). The fever began to fall on the 36th day and temperature became permanently normal on the 41st day.

Table VI.

IMPROVEMENT IN THE LEUCOCYTE COUNT AFTER TIN TREATMENT.

		Day of disease.	
		31st.	40th.
Haemoglobin	... ...	55	50
Erythrocytes	... .	3,850,000	3,550,000
Leucocytes	... .	4,200	5,600
Neutrophiles:	band forms ...	15 per cent.	12 per cent.
"	segmented forms...	58 "	40 "
Lymphocytes	... ...	26 "	45 "
Mononuclears	... .	1 "	3 "
Eosinophiles	... ...	0 "	0 "

The stimulating effect on the leucocytes of the therapeutic doses of tin, quite at variance with the depressing one of large doses, may contribute to its favourable action, but this question remains to be investigated further.

Besides the 100 acute cases we had the opportunity of treating two typhoid carriers. Here, the result should be achieved, if at all, still more slowly, as the tin accumulated in the intestinal lymphatic system would be of no avail, and only the deposit in the liver which is eliminated through the bile ducts might have an effect.

Case 3.

Malka R., female, 28 years old, suffered in June, 1940, from an undiagnosed fever, which lasted about 3 weeks. One year later she became cook in a collective settlement where she had to cater for fifty-five persons. Another sixteen inhabitants of the

same settlement suffering from various digestive troubles got their food from a dietary kitchen where another cook worked. In the second week of October, 1941, thirty persons out of the fifty-five fell ill with typhoid fever while all the sixteen dietarians were unaffected. It was found out that all the patients had eaten, about 2 weeks ago, from a milk pudding which had been left standing for 2 days. These facts drew the attention to the personnel of the general kitchen and stool examinations of the cook revealed the presence of *B. typhosus* in abundance ; no bacilli were found in the urine. Stools of this carrier were now examined three times weekly as from 19th October to 30th November and were always found to be positive. From 11th to 21st November, 1941, she was given the usual course of tin treatment and, as the stools were still positive after it, she took twenty tablets daily from 25th to 27th November, and ten tablets from 28th November to 9th December. On 1st December stools were for the first time negative and subsequent daily examinations, to 17th December yielded the same result. Up to 1st June, 1942, stools were regularly examined twice monthly and no further positive result obtained.

One may object against this case that it is not known how long the person was a carrier before treatment was started. It may be that her fever of the preceding year had nothing to do with typhoid, and that she acquired the bacilli only a short time before they were found. Then, her carrier state may have ended by itself after some months' duration without having been affected by the treatment. The next case, however, does not leave any doubt in this respect.

Case 4

Menahem B., male, 43 years old, suffered in July, 1931, from a fever which lasted about one month and which was diagnosed, probably on clinical grounds, as paratyphoid. Since then he was always in perfect health. When a typhoid epidemic occurred in October, 1940, in the village where he lived, stool examinations of all inhabitants were made and he was found to be a carrier. Examinations repeated from now on twice monthly always revealed the presence of *B. typhosus* in his stools. On 15th January he was admitted to our hospital for treatment of his carrier state. He took forty tablets daily from 16th to 21st and from 26th to 31st January, and ten tablets in the following periods : 9th to 11th February, 17th to 24th February and 28th February to 6th March.

As daily stool examinations remained invariably positive the whole time, he was discharged from hospital and continued treatment at home with ten tablets daily for a week alternating with a week's pause up to 21st May. Stool examinations carried out weekly were all positive. From 21st May to 22nd June he interrupted the treatment, but on 16th June stools were for the first time negative. From 22nd June onwards he again took ten tablets daily for 2 weeks, and all stool examinations up to now (15th July, 1942) have given negative results. In spite of the very high initial doses and the prolonged administration the patient never complained of any inconvenience.

In this case we are sure that the carrier harboured the bacilli at least one year and a half before treatment was started. It took, however, 5 months before he was freed from them.

We had no opportunity to try the tin treatment in paratyphoids.

Therapeutic animal experiments with plague-infected white mice yielded no results. As we have to deal here with a rapidly progressing septicaemia the higher susceptibility of *P. pestis* to tin obviously does not compensate for the lack of a common site of accumulation, which would bring about an intimate contact of tin and bacilli.

III.—Summary and Conclusions.

1. Metallic tin has a slow bactericidal effect on certain micro-organisms, amongst them *E. typhosa*.

2. A drug containing tin stearate and colloidal metallic tin had a favourable effect in typhoid fever, decreasing the death rate to 3 per cent. in 100 selected severe cases, while the average death rate of a control group of 428 patients including the mild cases was 8·2 per cent. It is very probable that the duration of the disease is shortened and that in a certain number of cases the toxaemic condition is improved. We are inclined to attribute these effects to the rather unique coincidence that drug and causative organism have a common place of accumulation, namely the intestinal lymphatic nodes. But it may still be open to discussion whether the action of the metal there is as simple as we supposed it to be, or whether it is rather of an indirect nature, consisting in a stimulation of cellular antibacterial activity.

3. The earlier treatment is started the better seem to be the prospects of success.

4. Two healthy typhoid carriers were treated with the same drug and lost their bacilli after taking it, with intermissions, for three weeks and five months respectively.

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TRANSACTIONS
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AND HYGIENE

VOL. XXXVI. No. 6. MAY, 1943.

ORDINARY MEETING
of the Society held at
Manson House, 26, Portland Place, London, W.1,
on
Wednesday, 24th February, 1943, at 4.30 p.m.
THE PRESIDENT
Sir RICKARD CHRISTOPHERS, C.I.E., F.R.S., COL. I.M.S. (ret.),
in the Chair.

PAPER.

SOME MEDICAL PROBLEMS IN THE COLONIES IN WARTI

BY

A. G. H. SMART, *C.M.G., M.B.E.*, M.D., D.P.H.
Medical Adviser to the Secretary of State for the Colonies.

I am glad to have this opportunity of telling you something of wh going on in the Colonies during the war. I should have liked to have this earlier but it is difficult, even at this stage, to say a great deal about is happening in the Colonies in wartime. However, recent developn have made it possible to be a little more expansive.

I cannot pretend that I am going to give you anything in the natu a scientific talk. In the main this will be simply a narrative covering aspects of medical work in the Colonies. But perhaps you will be able t some scientific bite later on when we come to discuss one or two of the prob that are specially prominent, for example, malaria and perhaps yellow fev

For convenience I am using the single word "Colonies" to includ those countries, whether they are called Colonies, Protectorates or Manc Territories for which the Secretary of State for the Colonies is responsibl

Difficulties Created by the War.

As might be expected, difficulties and problems have arisen out of the present struggle, and the impacts of the war itself have often made it not an easy matter to deal with these difficulties and the situations arising therefrom.

Perhaps we are all inclined to compare the difficulties that are being encountered during these times with those met with in the last great war, and possibly even with those that have arisen during earlier wars! But in this instance our problems have been magnified by the tremendous scope of the present conflict and by the vastness of the numbers involved. As the bulk of the Colonial Empire is sited within tropical or sub-tropical zones we shall, in the main, be discussing problems associated with this environment and setting.

Broadly, how have our difficulties arisen and what is their nature? From the personnel standpoint they are associated mainly with the fact that in war time troops and other people go to intrinsically unhealthy places and that they themselves have no acquired immunity to tropical diseases, and usually no previous experience of a tropical climate. Thus they are not knowledgeable in this sense and are unable to look after themselves properly. This at once creates a situation which is potentially dangerous and it may be aggravated by the fact that sometimes it is impossible, for security or other reasons, to make adequate preparation from a health standpoint for such people before their arrival. In addition to troops, which constitute the main group in this category, there may be persons shipwrecked through enemy action, landed on some territory. Furthermore, there are the civilian personnel which the exigencies of war have created, who are additional to the ordinary peace time establishments. A little consideration of the territories to which such people have gone at once suggests possibilities for the dissemination of disease.

While under peace conditions most of these territories can be reasonably controlled from the disease standpoint, the situation becomes at once altered with the imposition on these territories of what may be termed emergency factors.

Another important matter is the great increase in rapid transit that has arisen out of the war. Not only is transit rapid but people are being constantly moved from one area to another. There may be persons that are in some respect infective and hence constitute sources of disease. Thus areas that formerly were isolated, or comparatively isolated, either because of limitations of transport or for other reasons, have, during the war, been thrown together in one tremendous huddle. This shrinking of space applies not only to territories within a regional group but to continents. We know that now it is a mere step from the African continent to the American continents and the Caribbean Islands. Formerly the South West Pacific territories were comparatively isolated. Many of them were free from malaria and, to a very great extent, from other epidemic disease. They are now no longer isolated and are exposed

to such risks either through material or persons carrying infection by sea or by air.

The war has not only imposed difficulties but in itself has created difficulties in dealing with our problems. For example, there is the question of man-power that permeates everything and, as the war goes on, it is becoming increasingly hard to find people with special experience for certain jobs.

Today I can touch only on the fringes of the effort of the medical staffs in the colonies. I hope that some day the story of what the Medical Services have done in the common cause in the colonies will be recorded.

This is the first occasion during the war that I have had the opportunity of talking on this subject and I may be permitted to pay a tribute to the personnel of the medical establishments in the Far East, many of them members of this Society, who are isolated from us today under a foreign control. Although up-to-date news from Malaya and Hongkong is very scanty we know that, long before the final issue, the civil medical establishments were quietly going ahead with preparations to meet any emergency that might arise. For many months before Singapore fell the medical authorities had been organizing a blood transfusion service, and other emergency services, which were of the greatest value when the occasion arose. In both these territories we know that the ancillary medical services, especially the nursing staffs, carried on devotedly during the latter days. No doubt some day we shall know the complete story of their work then, and subsequently.

At the beginning of the war, or soon afterwards, many medical officers in the Colonial Medical Service were seconded or released for military service. In Africa alone the total amounted to something approaching a quarter of the total establishment. Many of those released were officers with specialist qualifications or experience.

Not only was this the case but much additional work has been imposed on remaining staff. Leave has been restricted both by limiting the amount of leave granted and by prolonging the interval between leave periods. This applies to all Government personnel. Later on I shall make some comment on the cumulative effect of these factors on staffs generally in the colonies.

While a good deal of attention must naturally be focused on the African continent because of the vast operational scale of the war effort there, similar problems have arisen in most territories of the Colonial Empire, for example, in Malta, Palestine, Aden, Ceylon, Mauritius, Seychelles and the Western Pacific. The likelihood of such problems arising under the abnormal conditions of the war was apparent. It is the degree in which they have arisen and the vulnerability of each territory that matter so much.

Special Problems.

I can touch only on some of the problems that are outstanding and on the way in which they have been met. It is not easy from this end to envisage the

immensity of the problem of health control under war conditions and the vast extension of effort that has been invoked by the war. Perhaps I might say at once that the position in relation to any territory in which military operations are going on is one that has to be shared by the civil and military authorities, although, in the ultimate issue, the former must be responsible for the state of the public health in any given territory.

In this connection liaison and co-operation between military and civil authorities has never been happier or more complete. Where Allied countries are concerned there is also close co-operation. Our American friends have been working with us in all the territories in which we are mutually interested and, in spite of the strain of the war on staff and resources, the Rockefeller Foundation have continued to give us their valued assistance and co-operation for which we are all most grateful.

The main likely problems spring to mind at once. They are to a large extent associated with the intimate contacts created by air travel and, generally, by the movement of people from one place to another.

Yellow Fever.

Travel in the ordinary way by sea, land or air is governed by International Conventions. But things have moved so fast in these latter years that the Conventions are largely out of date. They may be otherwise incompatible with the war emergency. Look for a moment at the *International Convention for Aerial Navigation*, framed in 1933. Considerable scientific advance concerning tropical diseases has been accomplished since then. For instance, where yellow fever is concerned, there is no reference in the Convention to modern accepted methods of pathological diagnosis, or to protection by inoculation.

The implementing and co-ordinating of the measures laid down in the 1933 Convention largely depended on the active and effective assistance of the Office International d'Hygiene Publique in France; it would also have initiated any steps for later revision of the Convention. But this body as an acceptable agency for co-ordination and control, disappeared with the partial occupation of France in 1940, and the question arose as to how the dangers of the spread of disease, so greatly accentuated by war exigencies, were to be countered. The military authorities were particularly concerned about this, and so were the colonies. Air routes were spread over the length and breadth of the African continent and it was closely linked up with the Americas on the one hand and with India on the other. Therefore it was essential that something should be done to bridge the gap that existed between the promulgation of the Air Convention in 1934 and the position today. The urgency of the problem was underlined by a serious outbreak of yellow fever that occurred in the Anglo-Egyptian Sudan in 1940-41.

Accordingly an Interdepartmental Committee was appointed to study the

public health problems of aerial travel in relation to war activities and primarily to try and co-ordinate the control of yellow fever between all the departments concerned, including the Services. They drafted an Interim Report at the end of 1941.

This Report took account of such important matters as the early detection and correct diagnosis of yellow fever, protection of all travellers within, or passing through, endemic areas, quarantine measures, effective disinsectization of aircraft, the control of *Aëdes* and other potential mosquito vectors of yellow fever, special measures in respect of aerodromes and research into yellow fever problems. The Committee took into account the advances in scientific knowledge since the ratification of the Aerial Convention and the transformation in aerial travel that had arisen through the war. They suggested that the recommendations in the Report should form the basis of yellow fever control and regulation. The Report was not distributed generally but was circulated to everyone concerned through the various departments. It seems to have been welcomed and the recommendations have been adopted, with slight modifications in some instances, by all colonial governments and generally by British territories.

I might here refer to some of the recommendations in the Committee's report. The desirability of people going into yellow fever endemic areas in Africa, as well as non-Africans residing in these zones, being inoculated is emphasised. To further this aim vaccine distributing and testing centres have been established in East and West Africa. The wide distribution of vaccine on the African continent and indeed elsewhere in the British Empire, has been made possible through the close co-operation and assistance of the Rockefeller Foundation, who have supplied millions of doses to Africa alone. The Wellcome Research Institution also come into this and have been looking after Colonial Office personnel for a number of years. They have carried out much research into the question of the development of immune bodies after inoculation. The Institute of Medical Research at Johannesburg were invited to take up the production of vaccine and have agreed to do this. Through the good offices of the Rockefeller Foundation their staff have been able to gain experience of the special technique of production in the U.S.A.

In all these matters the closest co-operation has been maintained between civil governments and the fighting services, as well as with everyone else concerned. The Interdepartmental Committee had representatives of all the Services and all the departments interested. Since the publication of their original Report another Interim Report has been produced, which includes amendments embodying the results of experience gained since the original Report was drafted.

With regard to the co-ordination of yellow fever research the recommendations of the Committee have been met by the action of the Rockefeller Foundation in re-establishing a branch laboratory at Lagos. The West African Governments are assisting financially and by providing accommodation. Thus

Dr. A. F. MAHAFFY, of the Entebbe Yellow Fever Laboratory, is now in general charge of yellow fever research in Africa. The Committee stressed the need for further research on the vectors of the disease and on possible reservoir hosts.

They recommended the appointment of an officer to inspect aerodromes within a certain area in Africa and a medical entomologist of the Colonial Medical Establishment was deputed for this duty.

For the purposes of quarantine control and inoculation the Committee recommended the establishment of *blocs* in Africa which should be regarded as endemic for these purposes. Thus control and disinsectization is concentrated at aerodromes outside the endemic *blocs*. Elsewhere the definition of "endemic area" is designed to include any region in which yellow fever exists, or has existed during the past 15 years, in a form recognized clinically, biologically or pathologically. This covers the period during which scientific knowledge has enabled diagnosis to be established on a firm and sure basis.

Although much more could be said about this important subject, including some reference to yellow fever in recent times, I am afraid that the ground that has been covered must suffice for this short review.

MALARIA.

I shall confine my remarks on malaria chiefly to what has been happening in Africa, although it is a special problem in many other territories.

In reviewing the position, perhaps I might run over some of the factors that have contributed to create a situation that is dangerous. In Africa the mosquito factor is one of first importance. *Anopheles gambiae* is easily the most important vector and is particularly dangerous because the conditions needed for its propagation are to be found so readily anywhere in tropical Africa. It is not a highly specialized breeder. Any casual collection of water that has been standing for a week or two suffices as a breeding place, as well as more permanent collections of water.

This might seem to make it a simple matter to control the breeding of these mosquitoes compared with that of more sophisticated mosquitoes with rather more selective habits. But the ubiquity of such sites and the ease with which they spring up create a big problem. Before the war this was hard enough but during the war difficulties have been many times multiplied.

The Secretary of State appointed a Mission, consisting of Prof. D. B. BLACKLOCK and Dr. CARMICHAEL WILSON, to go to Sierra Leone in 1940 to examine the problem on the spot. In the environs of Freetown they found certain areas, for instance at Kissy, where *A. gambiae* was breeding almost universally. Special efforts had been made during the last war to clean up this area, and it would have been better if these had been subsequently fully maintained and permanent measures of control developed. Some measure of control was maintained at Freetown, but this was entirely inadequate to ensure any proper measure of protection under conditions created by the war.

What has had to be considered was what was needed now (and for at least the duration of the war) with a scheme to ensure that permanent improvement at Freetown would be achieved. As well as *A. gambiae* itself, there is a variety (*A. gambiae*, var. *melas*) which, by virtue of its breeding habits, is perhaps an even more dangerous mosquito. The permanent work now being planned includes measures to deal with this mosquito which breeds in brackish water.

It is hoped that its measure will be taken by Dr. MUIRHEAD THOMSON, who has been sent to West Africa by the Colonial Office to study the habits and bionomics of *A. gambiae*. His studies may throw some light on the habits of the species and hence reveal possible new methods of control. Already his investigation is beginning to show useful results because, while the adults cannot always be differentiated, he has found a definite character for *melas* in the egg.

No one accepts the idea that there is any inevitability in acquiring malaria in West Africa, or elsewhere. If it is impossible to get control of breeding places by recognized methods, then other and newer ways will have to be devised. The control of casual water is largely a matter of routine sanitation and breeding will diminish with improved sanitary control. *A. gambiae* has been eliminated by the Rockefeller Foundation from Brazil and this goes to show that the problem in Africa, although immense, is not insuperable. Valuable work in mosquito control had been accomplished in Africa before the outbreak of war —for instance at Dar-es-Salaam and Mombasa.

In the consideration of this problem everyone concerned has been brought into the picture. It is not my place to tell you what has been done by the military authorities, nor would it be possible to do so at this stage of the war. But I can say that in all measures that have been taken, action for the common good is being assured by a coalition of forces. In each of the British territories in West Africa there are now territorial Committees who view the situation as a whole, while the Civil and Service authorities deal with the areas under their respective control. All these operations involve the assistance of specialist people who may be civil or military personnel.

No question of finance is allowed to stand in the way of measures that are considered necessary, but there are so many factors involved that it is difficult, under existing conditions, to envisage a complete solution of the problem. Adverse factors in West Africa summarized are: the imposing on an intrinsically unhealthy area of vast numbers of people who are unused to the tropics and have no acquired immunity to malaria, the universality of the main mosquito vector and its breeding habits, the increased liability to individual infection under Service conditions, as compared with peace-time conditions, the difficulty in obtaining as quickly as possible adequate amounts of materials such as mosquito netting and insecticides, the relative shortage of persons with the necessary training and experience to advise, and, finally, the lack of any complete or wholly satisfactory preventive measure by chemotherapy.

Malaria epidemics are a matter of getting a suitable combination of things, that is, the right temperature, the adequate prolongation of a certain degree of temperature, the presence of infective material in the shape of gametes and an adequate number of breeding places. In Northern France, during the last war, malaria occurred amongst the troops. There, however, the proper combination and synchronization of factors were lacking and the problem was never a really serious one, although the main carrying mosquito, *A. maculipennis*, was practically universal: it could be recovered from almost any shell hole in the Ypres area, in addition to other sites. But in West Africa all the necessary factors were supplied. There in the pot you had all the ingredients for trouble; all that was needed was someone to put a match to the fire.

Special groups of the population affected during war time include, of course, military personnel. Sometimes they are sited in areas where it is exceedingly difficult to maintain effective mosquito control, whatever staff is available for the purpose. For example, the control of *A. gambiae*, var. *melas*, must depend on a sound knowledge of its bionomics and breeding habits, and this is not yet available. Again, people cannot always tuck themselves away under a mosquito net at night: they often have to be on duty throughout the hours of darkness.

Another group is merchant shipping personnel on ships touching at malarious ports. Under war conditions ships are apt to be held up at ports, and in this way there is ample opportunity to acquire infection. Reference has already been made to the position at Freetown where cases of malaria have constantly occurred amongst this group. This was noted quite early in the war and the Mission already referred to reported to the Secretary of State at the end of 1941. Their recommendations were made after a year's investigation on the spot, and many of them had been practised for months before the report was received. The recommendations included the inspection of ships, the widest possible use of propaganda, proposals for anti-malarial work, both temporary and permanent, and various other measures. The position at Freetown has now very considerably improved and plans are going forward for more permanent control. Recent information goes to show that now infection is coming from ports in West Africa other than Freetown, and special measures in this regard have been already taken. All departments concerned are working closely together to do everything possible; and one of the measures taken has been the establishment of Welfare Officers, with local Welfare Committees, at seaports.

Another group specially liable to infection from malaria includes people in transit by air, who are thus likely to transmit unaccustomed strains of parasite from one territory to another. Persons shipwrecked through enemy action are landed from time to time in malarious areas. Their arrival is usually unheralded, and thus it has not always been a simple matter to arrange adequately for their reception or to make provision to protect them from malaria infection. Experi-

ence has shown the necessity of having standing arrangements made to deal with such problems, where this is practicable.

Earlier I have referred to the lack of any reliable preventive or suppressive measure by chemotherapy. We know that quinine taken suppressively keeps the parasites below the level that would lead to an attack and modifies attacks when they do develop, but we know of no safe drug that can entirely inhibit an attack and render a person non-infective. The position with regard to the suppressive use of drugs is one open to argument, but in West Africa the European-derived population has come to rely very much on taking quinine in this fashion. Probably the war, with its impacts, will have the effect of altering the situation very considerably from the West African angle. No doubt we can expect to see much more organized and systematic malaria control and, perhaps, as a result of the investigation now being undertaken, we may attain to the knowledge of a more effective and economical measure of control based on the habits of the mosquito. Furthermore, in time there will be much better education regarding the origin of malaria and its control, and hygiene will generally be brought to a higher standard. Possibly, too, wider and more systematic use will be made of mosquito screening and protection. In the East African group this measure has predominance over suppressive quinine.

The extensive work at present being undertaken by the military authorities in Africa is to a large extent of a "duration" character, and, in course of time, the Civil authorities will have again to assume full action in general mosquito control in West African territories.

Although emphasis has been laid on the increased chances of malaria outbreaks occurring in war time, there is the other side of the picture. The altered situation, coupled with the fact that the authorities are working together, has made it possible to secure more than ever before co-ordinated control in regard to the propagation of both yellow fever and malaria. This, in the nature of a post-war credit, should help us later on.

Apart from the African Continent, this danger looms fairly large in other parts of the Empire. The danger of the occurrence of malaria depends very much on how favourable a place is made for mosquito fauna, and on mechanical interference with drainage and normal water tables. This is bound to occur in war operations and constitutes a real source of danger from the vector standpoint.

Similar situations have arisen in other parts of the world through these factors. In the Far East the vectors are not all so simple-habited as *A. gambiae* itself. The main carriers in Malaya are two which have rather specialized habits, and those which predominate in other Far Eastern territories all have habits that need to be known to be thoroughly appreciated. But certainly interference with water tables and drainage would at once create a dangerous situation from this angle in territories that are malarious, this danger varying to some extent according to season and with the habit of the mosquito.

While this is so, and malaria must be a factor very much to be reckoned with in planning military operations, we know that these risks apply as much to the enemy forces as to our own. In the case of the Japanese, who are largely non-immune to malaria, the risk must be a very real one.

In addition to all this there is the risk of the spread of malaria by the agency of rapidly moving aircraft traffic importing into hitherto immune areas malaria-carrying, and possibly malaria-infected, mosquitoes. I have already said that the Western Pacific territories constitute such an area, and other epidemic disease may be imported as well, either by air or sea borne traffic. You may be sure that the authorities are fully aware of this possibility, and that everything possible is being done to minimize the risk of this occurrence.

Other Diseases.

Disease has also in war time the chance to propagate more readily through finding a favourable nidus on account of lowered resistance through privation and adverse sociological conditions induced by the war. This will be the picture so far as Europe is concerned. But, in regard to the Colonies, the state of nutrition and well-being has been maintained generally at a satisfactory level, although there have been from time to time various food shortages.

In some territories, however, war impacts have had adverse repercussions on the population. Malta has been practically in a state of siege since Italy came into the war. Here the death-rate amongst infants, and generally, has increased. This is due to unsuitable living conditions, some of the time in shelters, and to impaired nourishment.

TUBERCULOSIS.

We have evidence that more tuberculosis is being seen in colonial territories and fresh infection may be introduced in some cases by African personnel invalided on this account from the Forces.

TYPHUS.

The introduction into some territories of louse-borne typhus cannot be put aside. The movement of refugees into colonial territories might lead to epidemic outbreaks and this must be safeguarded against by measures of disinfestation and control. You are doubtless aware of the considerable extent of such movements during the war. There is also the danger of the transmutation of endemic murine typhus into louse-borne epidemic typhus.

VENEREAL DISEASE.

A rise in the incidence of venereal disease can always be expected in times of war, and to you I need not say very much about the factors that favour such an increase. The fact that this increase is mainly noted in our seaports in the

Colonies at once gives an indication of the influences that matter. Anyone who has had the opportunity of moving about in recent years, and this privilege has been somewhat restricted, cannot fail to have been impressed with the change that has come over some of the harbour areas in the Colonial Empire. The rise in incidence of these diseases is inevitable, having in mind the massive scope of war movements, and the combination of circumstances mainly responsible for the problem. The authorities concerned have had it very much in mind and are doing what they can to counter the conditions that favour such an increase. Measures must follow the usual lines, but in dealing with conditions in the tropics it is specially necessary that measures should be appropriate to the territory concerned and to the situation as it exists. Propaganda must be employed but it must bear a clear relationship to the outlook and mentality of those for whom it is intended. Thus propaganda suitable for people from the United Kingdom would not be appropriate for an indigenous population in West Africa. Welfare facilities and amenities are being provided; without these there is bound to be trouble. It is no good expecting people to keep clear of brothels unless one is prepared to look after their comfort on arrival at a port and provide some sort of entertainment and comfort. This problem has received anxious consideration at this end from all the departments concerned, as well as by the various Governments. It has serious repercussions on a transitory population but very many also on the indigenous population in the areas concerned.

Probably one direction in which something further could be done, is by better and more explicit education at this end. We are all very glad to note the dawning of a saner attitude towards this question at home. Our people going abroad have always been hampered by the point of view in the United Kingdom and this has not made the problem any easier for Colonial Governments to handle: they view this as part of the wider problem of social welfare; it is receiving special consideration at the Colonial Office.

CEREBROSPINAL FEVER.

Cerebrospinal fever is a disease that has lately become rather more prominent in some territories. It may be that a large amount of the increase is more apparent than real because, in the past, it has not been possible to get at all the cases of this disease. But, on the face of it, and viewing only figures of cases, *e.g.*, in Tanganyika Territory during the past few years, it is evident that there has been a considerable increase in reported cases. A satisfactory feature, however, has been the lower death-rates that have been seen. This reduction in the death-rate compared with the high death-rates in the past has been largely brought about by enlightened knowledge which has made it possible for cases to be seen earlier than before, and so early treatment with sulphonamides, in at least a proportion of cases, having become possible.

TSETSE FLY CONTROL.

Another problem that demands a great deal of attention is that of tsetse fly control in Africa. Several departments are concerned in measures of fly control and although funds have always been forthcoming for any measure that seems to be justified, or for any research that might give promise of throwing light on dark places, we still have anxious problems of human and animal trypanosomiasis facing us, or suspected. It looks as if the time has come when we should try to take stock of the position as a whole, and decide what action need be taken to ensure that the greatest benefits will result from the efforts of those who are devoting their thought and energies to this problem. The question, in its widest aspects, is having careful consideration from this point of view.

TSUTSUGAMUSHI DISEASE.

This disease, known by various other titles such as "tropical typhus," "scrub typhus" and "Japanese river fever," has caused trouble during certain stages of the various campaigns. In view of this, investigation has been going on for the last year or so into the possible production of a protective vaccine against the disease. Dr. R. LEWTHWAITE, the acting Director of the Institute of Medical Research at Kuala Lumpur, has been carrying out this investigation under considerable difficulties. In the first place he was deprived of guinea-pigs, rabbits and the eggs he wanted, by the action of the Japanese who were bombing Kuala Lumpur at the time of the initiation of this work (which had been suggested by the Medical Research Council). The work was thereupon transferred to Singapore and continued until nearly the end of January, 1942. Then he flew to Australia, taking with him invaluable material in the shape of guineapigs and rabbits variously infected with strains of the tsutsugamushi disease. The F.M.S. Government granted funds for the expenses of this investigation, which is now being continued at the Commonwealth Serum Laboratory in Melbourne, with the willing and wholehearted assistance of the Australian authorities. An interim report has indicated that the investigation is going on satisfactorily and that there is some promise of a favourable result.

NUTRITION.

In the years just previous to the war the question of malnutrition in the Colonial Empire had been receiving a great deal of attention and much information on the subject was being collected. In accordance with the recommendations contained in the *Report of the Committee on Nutrition in the Colonial Empire*, published just before the war, Dr. B. S. PLATT, of the Medical Research Council, had been directing a survey in Nyasaland to determine the state of nutrition in certain selected areas. The account of this survey, which was commenced in August, 1938, should serve as a model to direct

surveys amongst primitive peoples in the future. Dr. PLATT has pointed out how necessary it is to take into account all the various aspects of native life and custom, conditions and variations of season and rains, sociological habit and practice, and the impacts of extraneous conditions on a hitherto untouched population. It was intended originally that this survey should form part of an investigation for the whole of East Africa and that, after this was completed, Dr. PLATT would be able to visit other colonies.

The food factor, both in relation to supplies and distribution, has been a most important one, affecting well-being and the performance of work. The Government of a territory cannot know how they stand in regard to foodstuffs if production is unknown. Not only does this apply to staple crops but to secondary crops and all those small dishes that have so great a menu value in preserving a balance of nourishment. This latter factor is an important one not only in securing that the indigenous worker will take an adequate meal but also in its effect on effort and well-being.

Clearly, then, one of the first things to do—and this was done in this country—was to have a survey of food stocks made and capacity for production ascertained in all territories. This is being done and action is being taken by Governments on this information to increase, or to substitute, crops so as to secure the best advantage for everyone. It is difficult, however, to obtain a record of production of crops other than staple crops and, if this is hard to achieve in the United Kingdom, there must be even greater difficulty in colonial territories. As a result of what is being done under the stimulus of war exigencies, many territories have become largely self-sufficient. There is as well a much greater tendency to make the best use of local resources in every direction and to examine the value of local products. Perhaps this is one of the directions in which benefit and progress has come through the impacts of war.

It does not appear that any territory has suffered specially from malnutrition on account of the war except where enemy action has for the time being upset the actual import of foodstuffs, or economic conditions have been seriously affected in other ways. In many territories the cost of living has gone up and there has been a limitation of foodstuffs, with rationing. Much has been done to educate people as to the best ways that articles in short supply can be employed or substituted so as to secure a proper balance of nutrition. Much more in this direction is necessary and there is a valuable field of investigation ahead in the examination of local methods of processing foodstuffs, and their employment.

With regard to territories that come within the category of those that have suffered from enemy action, Hongkong had to endure something like 17 days of siege conditions before its fall on Christmas Day of 1941. This period would not have been long enough to produce any gross evidence of malnutrition. As yet we are unable to form any clear picture of the position related to subsequent events.

On the other hand, Malta has been under siege conditions since the middle of 1940 with the population depending very much on supplies introduced from outside. Although Malta's investment during this war did not preclude the possibility of providing the sustenance needed for maintaining life, many of the foodstuffs to which the inhabitants had been accustomed were cut off and nutrition was upset very materially. Nevertheless, in late 1942, it was not possible to detect much evidence of gross malnutrition amongst the ordinary people in Malta. There had been adverse repercussions, seen in the heightened death-rates and some increase in tuberculosis and other disease. But evidence of malnutrition was confined to less apparent findings, and perhaps to a much lower state of resistance to disease. Things had not then attained a stage likely to have produced permanent results, or one which could not fairly rapidly right itself when better conditions ensued. This has now, fortunately, happened, and we shall in due course know more of the effects of the ordeal on physical health. Much of the unexpectedly favourable state of the Maltese population after these trials must be ascribed to their own gallant and cheerful disposition, which refused to allow material conditions to become a predominating factor.

It has been possible to make a good deal of progress in public welfare in the British West Indies during the last two years, in spite of war preoccupations. Sir FRANK STOCKDALE's organization has been quietly working there garnering knowledge of the various problems and sounding and influencing public opinion. Sir FRANK STOCKDALE is Comptroller of the funds allocated under *The Development and Welfare Act*. His organization has been implementing the work and recommendations of the Royal Commission which visited the British West Indies in 1938-39. His Report has just been published, and he is able to tell us of a considerable revolution of agricultural method and of public health development, giving promise of a hastening of better conditions for the people. The bogy of finance has been largely removed and some co-ordination of action on right lines is being assured.

HEALTH OF THE UNITED KINGDOM-DERIVED POPULATION IN WAR TIME.

A prolonged period of war imposes severe strains upon European-derived populations in a tropical dependency. These effects are aggravated by climatic influences and by those of expatriation generally, with detachment from wives and children. The peculiar influences at work in the Colonies are not usually associated with life in the home country. In addition, leave is greatly curtailed in these days and periods of work have been prolonged. Much additional work has been thrown on available staff reduced on account of war demands. There is planning, not only for administration during the war but also planning for the post-war period. At the beginning of the struggle Colonies shared lavishly with both hands their available man-power resources and, after more than three years of war, they are feeling the result of this natural generosity.

There are other factors as well. People read about austerity measures and

plans in this country and want to undertake a proper share in self-denial and self-sacrifice. But austerity in a shade temperature of 95° F., with a correspondingly high relative humidity, together with the inexorable demands made by the tropics in so many ways, may constitute a burden that is intolerable. Civil servants, and others in the tropics, are diffident about disclosing a sense of tiredness or disability because they know that their temporary absence would impose an additional burden on their colleagues.

Passages to this country, and to the usual centres of recreation, are subject to priorities and at these places accommodation is at a premium because buildings and hotels have often been commandeered. Therefore, while it is desirable, essential indeed, to ensure that people must be able to recreate themselves at suitable intervals and get away from their job, arrangements—sometimes entirely fresh and novel arrangements—have to be planned to achieve this.

With all this it is necessary to cut down work to the limits absolutely essential to civil administration, and to the war effort, and to preserve a balance between present needs and future development. In doing this we are thus not only thinking of the war, with its ever-present demands, but of those difficult and exhausting years that will follow the peace and demand the employment of our best brains and manhood.

Medical Policy in the Colonies.

In this talk I have been discussing medical problems in the Colonies that have been prominent during the war, so far as it has gone. But the problems of war are, in the main, the problems of peace. The emergency has only underlined these problems, multiplied them many times, and increased the difficulties that there are in dealing with them. We have to carry on from peace to war and from war to the days afterwards. The impacts of the war on our Colonies will have many lasting results, none more so than those relating to the public health. The emergency period, and close contacts with medical people from outside, will have had a stimulating effect on the Colonies, and work begun or expanded during the war cannot be allowed to lapse, where it is conferring benefit on the population.

There is a much greater hope of getting ahead in the coming post-war years because advance in medical matters will not have to depend largely on the state of Government revenue in each territory. The institution of *The Development and Welfare Act* will help to ensure that funds will be available for this purpose. As I indicated earlier, a good deal has already been achieved in the last two years in the British West Indies and, while in other Colonies development has been held up to a very considerable extent by war exigencies and the over-riding demands of the war on personnel and material, something has already been accomplished. There has been planning on the right lines

and programmes relating to medical development are arranged to cover a period that will ensure maintenance of a scheme and continuance where necessary.

The Secretary of State's Colonial Advisory Medical Committee have recently drafted a *Statement of Medical Policy in the Colonies*, which has been accepted in principle by the Secretary of State. In this the importance of preventive measures, with a better knowledge of hygiene amongst the masses, has been stressed, as well as the need for training people appropriately for service in the various territories of the Colonial Empire. The statement suggests planning to bring all departments and people concerned into the picture, so that, for example, efforts to improve nutrition and reduce infant mortality will constitute part of a common effort to raise the standard of living. It generally brings medical policy in the Colonies up to date, and in harmony with what experience has shown are the most practicable ways of improving health conditions and raising the general standard of well-being.

All this should ensure that in the years to come medical development in the Colonies will be continued, if perhaps slowly in the more backward territories, on lines best calculated to achieve the most beneficial and enduring results.

Discussion.

Professor D. B. Blacklock: I was employed with Dr. Carmichael Wilson to investigate the malaria problem in Freetown, and it was somewhat complex. In the very early days Ross recorded that mosquitoes could be encountered on board ship, but I do not think he ever imagined you could have mosquitoes in such large numbers in ships so far away from the land as they were in Freetown harbour. That was a very interesting point. We were dealing with a harbour which had a very greatly expanded volume of shipping using it, as compared with pre-war days. It was suggested the anopheles got from the shore by lighters, barges and service vessels on to the ships, travelling the short distances from one ship to another. That did occur, but another interesting thing occurred also, to a much greater extent. Although the harbour was at its widest point 8 miles across and at its narrowest point about 3, in certain tornado winds it appeared as if the whole anopheles and other mosquito population of the villages on the other side of the estuary were swept across the harbour on to the Freetown side. There was a most remarkable invasion of the ships when the wind came from the Bullom shore. It was not an occasion to sit down to research on specific problems: we worked from hand to mouth and applied every method we could think of. We had great difficulty over the *melas* question, and in our reports we drew attention to the necessity of doing something about it. I was very glad to hear that an entomologist had been appointed to investigate, and that Dr. Muirhead Thomson is making useful discoveries about this *melas* variety; this has proved one of the most difficult things to deal with from the medical point of view, owing to its habit of breeding in saline waters among the mangroves.

As regards the sources of malaria we ought to keep our minds on the villages: if we remember that all disease comes from the villages it helps to give us a basis to work on. If you think of the villages as being the source of almost everything that matters in the way of disease you can begin to remedy this by village reconstruction. Many years ago (1932) I wrote a book, "The House and Village of the Tropics," which I offered to a publisher: he did not like the look of it and I published it myself. I was even then impressed by the fact that the reconstruction of the house, keeping the compound in order, and looking after everything in relation to the village was really the way to set about improving health in the tropics. As regards malaria, many of the villages we worked at had compounds which were simply bare patches of rock with holes. They had nice gardens laid out in the old settlement days, but after the first few years of rains their gardens were washed away and were lying at the bottom of the estuary, and they have been lying there ever since. There is nothing left but the rock. That is the sort of problem to be dealt with—reconstruction of village compounds and gardens; that in itself will greatly reduce breeding.

In our report we mentioned the diseases carried by mosquitoes—malaria (with its sequel blackwater), a couple of the filaria infections, yellow fever and dengue. We recommended that, instead of having one committee looking after blackwater, another looking after malaria, another after yellow fever and so on, the control of mosquitoes which carried disease should primarily be by a single large central committee which, according to the incidence of the particular diseases in the territory, would delegate to sub-committees work on the yellow fever mosquitoes or anopheles and so on. If we keep the picture before our minds of the unity of control of all mosquito-borne diseases it will help us with regard to prevention.

As regards the West Indies, a great deal of work is going on. Dr. SMART mentioned the West Indian Commission Report; is it going to be published in the near future? I gather that the work of Sir FRANK STOCKDALE and Sir RUPERT BRIERCLIFFE is going along pretty well on the lines of that report, and it would be interesting to see it.

I feel at the present moment a defender of the Colonial Office, although I do not always appear in that guise. Critics of the Colonial Office have been numerous, and I certainly think we are entitled, in our own professional matters, to express our critical views on every subject on which we are competent to do so; but I do feel at the present time a little local patriotism, because it seems to me to be the habit in foreign countries to talk about our Empire and Colonies in a very disparaging way, and I do not think one is bound to listen to that sort of thing indefinitely without saying something about it. There was the enactment called the *Statute of Westminster* in 1931 by which Canada, Australia, New Zealand, Newfoundland and the Union of South Africa became independent nations—completely independent for all practical purposes. What we have to

remember is this, that they were in the colonial status for years. If they had found the administration of the Colonial Office and its predecessors so distasteful and irksome, what would their reaction have been in the present circumstances? Do you think we should have found them sacrificing their men and money to keep together an imperial system which is as ramshackle and miserable as it is often said to be? I do not think so. All the colonies are on the road to the *Statute of Westminster*. Some are very near it, and others approach it more gradually. It used to be one of the great arguments against the Colonial Office that it believed in the policy of gradualness, and gradualness was described by hostile people as doing nothing, but doing it so gradually that you could not notice it. I will not take up any more time, and I wish in conclusion to thank Dr. SMART for his very interesting address.

Major-General D. T. Richardson: I would like first to say how very grateful I am to the Colonial Medical Services for having provided the Army with so many of the malariologists who have been, and are, doing such excellent work in the control of malaria in our armies in the various theatres of war.

In the tropics the disease problem, or at least 50 per cent. of it with which an army in the tropics is faced, is an entomological one. If we could eradicate the mosquito, the fly and the louse we would do away with much of the sickness that weakens an army in the field.

Not only are these insects carriers of specific diseases, but their bites are liable to become septic and give rise to a great deal of minor sickness. One-eleventh of our admissions to hospital in the Middle East are skin cases, a large proportion of which is composed of these septic conditions.

We are all sanguine that at no distant date we shall be leaving tropical areas and closing in on Germany where we shall be rid of the infected mosquito. To get there, however, we still have to pass through some very malarious areas.

The fly will, unfortunately, remain, though not in such numbers nor so virulent as its desert relative.

The danger of the louse will increase as we advance into Europe. It is our experience that in the mobile warfare of today we do not get nearly the same degree of infestation as we did in the last war when we were crowded together in trenches. Static warfare may, however, return, in which case the risk of infestation and of typhus will be greatly increased.

I would like to refer here to the excellent results we are obtaining in the Middle East by the use of Professor BUXTON's Sherlice belts in controlling infestation amongst native labourers. Despite the fact that these men return to their villages at night and are thus exposed to heavy infestation, it is the exception to find anyone with live lice on them. In the presence of typhus these men could not be regarded as a danger to the Army.

For the soldier in battle personal protection, whether against the mosquito or the louse, is the only practical proposition. We are doing our best to provide him with it.

Dr. Hugh Smith: As a representative of the Rockefeller Foundation, I should like to thank Dr. SMART for his generous words in regard to the work of our organization in the Colonies. During the period of approximately 30 years that we have been co-operating with the Colonial Office we have enjoyed the happiest relations not only with the Colonial Office in London but with dozens of Colonial officials and medical officers in the Colonies throughout the world. Perhaps it would be interesting to point out that the first international health work undertaken by the Rockefeller Foundation was in a British Colony: this was in British Guiana in 1914 when a co-operative programme for the control of hookworm disease was undertaken. In spite of the handicaps imposed by the war, shortage of personnel and other difficulties, we are still collaborating with the Colonial Office in Trinidad, Jamaica, British Guiana, East Africa and other places.

Among those who have never lived in the tropics, or visited tropical countries, there is a tendency to compare health conditions there with those which obtain in our own countries in the temperate climates such as Britain, Canada or the United States. Several speakers have already pointed out that there has been considerable criticism in recent months of the British Government in its handling of Colonial problems. But I, who have lived in a British Colony for 3 years, and visited five or six other colonies, feel that I have some claim to speak about the health work in the British Empire, and I must say that in my opinion most of the criticism has not been justified. One has only to visit the Colonies which are being governed by other countries, and to inspect the health work in independent tropical countries such as those of Central and South America, one has only to visit such places and compare their health standards with those of the British Colonies to see that a very praiseworthy standard has been achieved by the British Colonial officials. Naturally, there is plenty of room for improvement, but, as I said a moment ago, those who have not lived in the tropics sometimes do not have a real appreciation of the difficulties of health work in the climatic conditions which prevail in the over-populated regions that one finds in the tropics. One hopes that an effect of this war—and I feel sure there will be such an effect—will be a great stimulation of interest in tropical disease, not only more scientific research but a wider application of known data by the public health authorities. I feel that this Society ought to stimulate and can stimulate a great deal of interest in tropical medicine. It is a subject that has been shamefully neglected in America and certainly has not received the attention that is due to it here. Dr. SMART's address has called our attention to the complexity of the problems of tropical hygiene, and it will serve a very useful purpose in initiating discussion along those lines.

Wing-Commander C. J. Hackett: I would like to say how much help in West Africa the Colonial Medical Service gave the Royal Air Force. On all occasions the most willing and complete co-operation was received.

Dr. SMART's paper has raised the question of the position of anti-malarial measures in West Africa. In Sierra Leone work commenced during the last war was not, apparently, completed, and little appeared to have been done until the present war broke out. I do not know of any area in British West Africa which could be regarded as anti-malarial. This was in contrast to East Africa, where Mombasa was practically malaria-free.

In East Africa great use was made of and great value placed upon mosquito proofing of houses. In West Africa but little adequate proofing existed before this war and local opinion was against its use. These great differences in practice and opinion were very strange. Either mosquito proofing was of use and should be used in West Africa or it was useless and money should not be wasted on it in East Africa. Local conditions were not so different as to modify this extreme view.

The Aerodrome Inspecting Officer on passing through the capital of a large West African colony, on his way from East Africa, stayed one night in the house of a Senior Medical Officer. A fortnight later he developed malaria and his faith in suppressive quinine was considerably shaken. His experience was not unique.

Dr. Smart (in reply): Professor BLACKLOCK has spoken of research and the importance of treating problems relating to the transmission of disease as a whole, more especially in regard to mosquito problems and the diseases with which they are concerned. I don't know whether members are aware that there is now a Committee at the Colonial Office dealing with Research. One of its functions is to co-ordinate and guide research in the Colonies, and this will help to ensure that problems are treated in the widest possible way. With regard to the Report of the Royal Commission to the West Indies, I think that it has already been announced that the Report will not be published at present. But the Stockdale Report on Development and Welfare activities in the British West Indies during the past two years gives a clear picture of what is going on and what is being done to implement the recommendations of the Commission.

I am very glad to see Wing-Commander HACKETT here. I agree with what he says about some difference in the point of view between East and West Africa on anti-malarial practice. I think, however, that the effect of the great amount of movement that is going on nowadays, and contact with members of the Forces and others, will help to create a point of view more applicable to tropical Africa as a whole. This pooling of ideas will have permanent effects after the war.

I thank you all for your patient hearing of my address.

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COMMUNICATIONS.

THE REACTION OF THE AFRICAN GRIVET MONKEY (*CERCOPITHECUS AETHIOPS CENTRALIS*) TO YELLOW FEVER VIRUS.

BY
T. P. HUGHES,
*From the Yellow Fever Research Institute, Entebbe, Uganda.**

It is axiomatic in yellow fever epidemiology that epidemics, or epizootics, must result when infected haematophagous insects of a species capable of transmitting this infection by bite are present in adequate numbers among a susceptible population. To the present time some twenty species of mosquitoes have been proven capable of transmitting yellow fever virus by bite; of these twenty species some ten occur commonly in Eastern Africa, including several species which live in close contact with the human population. In the recent epidemic of yellow fever in western Uganda the infection was, presumably, transmitted to man by *Aëdes* (*Stegomyia*) *simpsoni* Theobald (MAHAFFY, SMITHBURN, JACOBS and GILLETT, 1942). In the Sudan epidemic of 1940 it is probable that *Aëdes* (*Stegomyia*) *metallicus* Edwards and *Aëdes* (*Diceromyia*) *taylori* Edwards were among the more important vectors (LEWIS). It is probable that any of these twenty species of mosquitoes would be capable of transmitting yellow fever virus in an epidemic form if infection was introduced from an outside source into a mosquito-infested and non-immune community.

* This Institute is supported jointly by the Medical Department of the Government of the Uganda Protectorate and the International Health Division of the Rockefeller Foundation.

Infection may be introduced into such a community from its hypothetical forest reservoir by any of three alternatives, singly or in combination: (1) by the entry of an infectious human; (2) by the entry of an infected insect; or (3) by the entry of an infectious animal. It is with one aspect of the last alternative that this investigation is concerned.

One of the most nearly ubiquitous animals in the East African zone of yellow fever endemicity is the grivet monkey, *Cercopithecus aethiops centralis* Neuman. This species, or monkeys of closely related subspecies (all belonging to the species *Cercopithecus aethiops* Linnaeus), occurs commonly in Uganda, in the Belgian Congo, in the Anglo-Egyptian Sudan, and in the remaining parts of the Ethiopian region. These monkeys are migratory and travel in small troops. Although primarily arboreal, they are often observed feeding or travelling on the ground. In many areas they cause annoyance and economic loss through their propensity to raid banana plantations, maize farms and vegetable gardens in search of food. If these animals were capable of circulating yellow fever virus in a concentration adequate to permit the infection of biting insects, a troop of grivets might distribute infection throughout its range of migration.

That infection of this species does occur in nature has been established by observations made by FINDLAY (1941) in Uganda and in the Sudan and, independently, by workers in this laboratory, that about 20 per cent. of these animals captured in each of two areas of yellow fever endemicity possessed yellow fever antibodies.

EXPERIMENTAL.

This investigation of the susceptibility of *Cercopithecus aethiops centralis* to yellow fever infection may be divided, conveniently, into three sections: (1) the reaction to pantropic virus administered "artificially" by injection; (2) the reaction to pantropic virus administered "naturally" by the bite of infected mosquitoes; and (3) the reaction to neurotropic virus.

In each case the plan of investigation was similar. The virus was administered, the animals were bled, usually daily, thereafter, and the sera so obtained were tested for yellow fever virus content by the intracerebral injection of mice with serial dilutions of the sera. Endpoints were calculated by the method of REED and MUENCH (1938).

It is unfortunate that monkeys of this species, when captured as adults, do very badly in captivity. Many animals died before being inoculated or shortly thereafter from nonspecific causes. Sections were prepared from the livers of all animals dying after inoculation, but of all the animals tested, only one, Monkey 22, showed lesions in the liver similar to those observed in rhesus monkeys dying of experimental yellow fever infection.*

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The temperature of all inoculated animals was taken twice daily but was so irregular as to be useless as a means of diagnosing infection. In general, fever seemed to accompany the presence of virus in the circulation but numerous nonspecific fevers also occurred.

REACTION TO PANTROPIC VIRUS ADMINISTERED BY INJECTION.

Ten monkeys were inoculated intraperitoneally with 1 ml. of Asibi serum virus in varying dilution and one with 0·5 ml. of rehydrated Asibi virus as shown in Table I.

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6	0	0	0	0	0		+				1 ml. Asibi 10[illegible]
7	0	0	+	+	+	Dead					1 ml. Asibi 10[illegible]
8	0	0	+	Dead							1 ml. Asibi 10^{-6}
9	0	0	0	0	0	0	0	0	0	0	1 ml. Asibi 10^{-2}
10	0	0	0	0	0	0	0	0	0	0	1 ml. Asibi 10^{-2}
11		0	0	0	0	0	0	0	0	0	0·5 ml. rehydrated Asibi
12	0	+	+	+	+	+	+	Dead			1 ml. Asibi 10^{-2}
13	0	+	+	+	+	+	+	+	Dead		1 ml. Asibi 10^{-3}
14	0	+	+	+	+	+	0	0	0	0	1 ml. Asibi 10^{-1}
15	0	+	+	+	+	+	Dead				1 ml. Asibi 10^{-1}
Monkey	**Day Following Mosquito Bite.**										**Mosquito Species**
	1	2	3	4	5	6	7	8	9	10	
16	0	0	+	+	+						*Aëdes (Stegomyia) metallicus*
17		0	+	+	+	0	0	0	0	0	*Aëdes (Stegomyia) aegypti*
18	0	+	+	+	+	+	0	0	0	0	,, ,, ,,
19	0	+		+	+	0	0	0	0	0	,, ,, ,,
20	0	0	0	0	0	+	+	+	0	0	,, ,, ,,
21				+	+	0	0	0	0	0	,, ,, ,,
22		+	+	+	+	+	+	Dead			,, ,, ,,

Circulating virus was demonstrated in the serum of eight out of the eleven animals inoculated. Of the three which showed no virus in the circulation, Monkeys 9 and 10 received the same virus suspension. This suspension was tested by inoculation into mice at the time when the monkeys were injected

Infection may be introduced into such a community from its hypothetical forest reservoir by any of three alternatives, singly or in combination: (1) by the entry of an infectious human; (2) by the entry of an infected insect; or (3) by the entry of an infectious animal. It is with one aspect of the last alternative that this investigation is concerned.

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10	0	0	0	0	0	0	0	0	0	0	1 ml. Asibi 10^{-2}
11		0	0	0	0	0	0	0	0	0	0·5 ml. rehydrated Asibi
12	0	+	+	+	+	+	+	Dead			1 ml. Asibi 10^{-2}
13	0	+	+	+	+	+	+	+	Dead		1 ml. Asibi 10^{-2}
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17		0	+	+	+	0	0	0	0	0	*Aëdes (Stegomyia) aegypti*
18	0	+	+	+	+	+	0	0	0	0	" " "
19	0	+		+	+	0	0	0	0	0	" " "
20	0	0	0	0	0	+	+	+	0	0	" " "
21				+	+	0	0	0	0	0	" " "
22		+	+	+	+	+	+	Dead			" " "

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18	0	+	+	+	+	+	0	0	0	0	" " "
19	0	+		+	+	0	0	0	0	0	" " "
20	0	0	0	0	0	+	+	+	0	0	" " "
21				+	+	0	0	0	0	0	" " "
22		+	+	+	+	+	+	Dead			" " "

Circulating virus was demonstrated in the serum of eight out of the eleven animals inoculated. Of the three which showed no virus in the circulation, Monkeys 9 and 10 received the same virus suspension. This suspension was tested by inoculation into mice at the time when the monkeys were injected

and it was found to be fully active. Serum taken from each of these two monkeys 21 days after inoculation contained no protective antibodies. The third animal, Monkey 11, received an injection of a rehydrated desiccated virus, a portion of which was injected at the same time into a rhesus monkey. The latter animal succumbed to yellow fever infection. No explanation is available for the failure of these three animals to circulate virus. Prior to inoculation they were bled, as were all animals used in this investigation, and their sera, when examined in the mouse protection test, were shown to be nonprotective.

Sera from Monkeys 5, 6, 7 and 8 were tested only for the presence of virus. The results obtained from daily titrations of circulating virus in the four remaining monkeys are shown in Table II.

TABLE II.

TITRE OF CIRCULATING VIRUS FOLLOWING ADMINISTRATION BY INJECTION.

Day after Injection	Lethal Doses for Mice per ml. Serum.			
	Monkey 12	Monkey 13	Monkey 14	Monkey 15
1	0	0	0	0
2	141	1,544	66	148
3	590,700	1,042,800	18,876	10,428,000
4	>3,300,000	>3,300,000	6,699,000	8,349
5	>3,300,000	>3,300,000	104	96
6	>3,300,000	23,265	85	Dead
7	1,042,800	1,042	0	
8	Dead	56	0	
9		Dead	0	
10			0	

It is apparent from these results that, once the infection is established, virus circulates for about the same length of time and in about the same concentration as it does in the serum of man or rhesus monkeys suffering from yellow fever infections. However, the reaction seems to be much more irregular and unpredictable than with the other primates mentioned.

REACTION TO PANTROPIC VIRUS ADMINISTERED BY MOSQUITO BITE.

Seven additional monkeys were subjected to bites by infected mosquitoes. These insects had been infected by feeding on a rhesus monkey during its first febrile period following injection with Asibi virus. Monkey 16 was bitten by four insects of the species *Aëdes (Stegomyia) metallicus*, the remainder were bitten by infected *Aëdes (Stegomyia) aegypti* Linnaeus.

The results obtained from the titrations of circulating virus are shown in Table III.

TABLE III.

TITRE OF CIRCULATING VIRUS FOLLOWING BITE OF INFECTED MOSQUITOES.

Day after Biting	Lethal Doses for Mice per ml. Serum.						
	Monkey 16	Monkey 17	Monkey 18	Monkey 19	Monkey 20	Monkey 21	Monkey 22
1	0		0	0	0		
2	0	0	1,650	247	0		33,000
3	10,428	7,788	1,042,800		0		3,300,000
4	28,100	1,042,800	1,207,800	183,150	0	688,100	13,200,000
5	104,280	7,854	115	33	0	33	330,000,000
6		0	33	0	1,471	0	7,788,000
7		0	0	0	1,666	0	Dead
8		0	0	0	216	0	
9		0	0	0	0	0	
10		0	0	0	0	0	

Although there is again a marked lack of uniformity of response, each of the seven monkeys showed virus in the circulation at some time and, with the doubtful exception of Monkey 20, in a concentration high enough to infect mosquitoes. It is of interest that Monkey 22, a very young and small specimen, had the highest concentration of virus in the serum and was the only one of the twenty-two animals used which showed, in liver section, pathological lesions similar to those occurring in rhesus monkeys following fatal infections.

REACTION TO NEUROTROPIC VIRUS.

Only one experiment using neurotropic virus, designed to demonstrate the absence of virucidal substances in the serum of this species of monkey, was performed.

Each of four monkeys was given an intraperitoneal injection of neurotropic virus. The virus used was in its 619th serial mouse passage. It contained, when undiluted, 144,540,000 lethal doses for mice per millilitre. Each monkey received an injection of 1 ml., the dilution being decreased by one hundredfold for each animal. The monkeys were bled daily. The sera so obtained were tested for the presence of yellow fever virus and also for yellow fever antibodies. The results obtained are shown in Table IV.

TABLE IV.

REACTION TO NEUROTROPIC VIRUS.

Day	Lethal Mouse Doses per ml. serum	Antibody
Monkey 1—Injected with 144,540,000 lethal doses for mice.		
Day	Lethal Mouse Doses per ml. serum	Antibody
1	0	0
2	0	0
3	68	0
4	265	0
5	0	0
7		
Monkey 2—Injected with 1,445,400 lethal doses for mice.		
Day	Lethal Mouse Doses per ml. serum	Antibody
1	478	0
2	4,356	0
3	10,395	0
4	1,947	0
5	59	0
7		
Monkey 3—Injected with 14,454 lethal doses for mice.		
Day	Lethal Mouse Doses per ml. serum	Antibody
1	0	0
2	0	0
3	0	0
4	[illegible]	0
5	0	0
7		
Monkey 4—Injected with 144 lethal doses for mice.		
Day	Lethal Mouse Doses per ml. serum	Antibody
1	0	0
2	0	0
3	1 221	0
4	775	0
5	75	0
7		

Each of the monkeys showed circulating virus at some time and all had become immune by the 7th day after inoculation. The experiment shows the absence of normal virucidal substances in the blood of these animals in two ways, by the negative results of the protection tests done with sera from early bleedings and also by the demonstration that yellow fever virus multiplies and circulates, even when injected in minimal amounts.

The test also demonstrates a marked variation in the response of individual animals, since there is no well-defined relationship apparent between the magnitude of the inoculum on the one hand and the length of the incubation period or the quantity of circulating virus on the other hand. This individual variation in susceptibility has been very apparent in all tests in which monkeys of this species were used.

Discussion.

The results obtained with respect to the reaction following the administration of virus by injection are similar to those reported by Theiler and Hughes (1935), who investigated the reactions of the closely related West African "green monkey," then referred to as *Lasiopyga callitrichus*, which under revised terminology becomes *Cercopithecus aethiops sabeus* (Linnaeus).

To date, about thirty species of primates have been tested for yellow fever susceptibility (Hindle, 1933), ranging from chimpanzees to lemurs. In many species the multiplication of virus has been experimentally observed. All species become immune following the injection of virus, a fact which may be interpreted as indicating that the virus has multiplied and circulated. Tests by Gordon and Hughes (1936) have shown that, at least with rhesus monkeys, no yellow fever immunity results without the multiplication of virus. It appears highly probable that all primates react similarly to the introduction of yellow fever virus in that the virus multiplies and circulates, but species differ greatly in their clinical response to the presence of this virus.

The limited number of primates, including man, living in any given area is probably insufficient to permit them to serve as a permanent reservoir of yellow fever infection, in view of the short duration of the period of circulating virus and the fact that the infection produces a lifelong immunity. It is, however, possible that the lower primates may serve as mobile distributors of virus, especially if the illness produced is not severe, by carrying the infection from the forest to an area infested by domesticated mosquitoes. In this manner primates may serve as one link in the chain of circumstances resulting, finally, in human infection.

Summary.

Certain individuals among the African grivet monkeys, *Cercopithecus aethiops centralis* Neuman, are capable of circulating yellow fever virus in high

concentration following its administration either by injection or by the bite of infected mosquitoes. There is, however, a marked variation in susceptibility among individuals of this species.

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PARAGONIMUS CYST IN A WEST AFRICAN NATIVE.

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AND

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The occurrence of paragonimiasis in Africa has not received general recognition.

Recent editions of Manson-Bahr (1941), Stitt (1939) and Blacklock and Southwell (1940) do not mention Africa in the geographical distribution of this disease. Craig and Faust (1937), however, state that the infection is endemic in the British Cameroons and the Belgian Congo.

Hegner (1938) includes Africa in his geographical list and E. C. Smith (1939) mentions that the disease has been known to occur in West Africa. The Annual Reports on the Medical and Health Services of Nigeria for the past 15 years record only two cases, both of which occurred in natives of the Cameroons and had characteristic ova in the sputum. A third case of lung infection in a Cameroons native was reported in a personal communication from Dr. W. C. Dale, Medical Officer, Nigeria, in 1939. A specimen of the sputum was sent for examination and the presence of numerous *Paragonimus* ova confirmed. In a further letter he made the interesting observation that no ova were found after a course of ten injections of fouadin.

The present case is recorded because of its interesting clinical features and to draw attention to West Africa as an endemic centre of the disease.

It is also of interest as showing that the endemic focus is not limited to the Cameroons.

* The authors are indebted to the Director of Medical Services, Nigeria, for permission to publish and to Mr. J. E. Knight, Laboratory Superintendent, for the photomicrographs.

CASE.

The patient, a well developed African male, aged 23 years, was first seen in September, 1942, complaining of a swelling in the neck. On examination a fluctuant swelling about 5×3 cm. was found in the upper third of the left sternomastoid, apparently in the muscle, painless and not associated with enlargement of lymph glands or any oral condition. There was no otitis externa and the drum was normal. Pus was aspirated and on examination was found to contain *Paragonimus* ova. On this report an exploratory operation was performed, a thin walled cyst being found deep to the sternomastoid in the muscle tissue of the levator scapulae and extending back to the base of the skull. Unfortunately it ruptured and could only be incompletely removed.

In the light of these findings a more complete investigation was carried out.

History.

The patient was born at Okarki in the Ahoada district of Owerri Province, Southern Nigeria, and had spent all his life there until 18 months ago. He stated that he was accustomed to eat fish, shrimps, snails, crabs, crayfish and lobsters which abound in the creeks and rivers of his home. These are not eaten raw but are roasted. (West African "roasted" means anything from a cinder to almost raw.) In 1931 the patient had a "fit" lasting about 2 minutes in which he fell over and struggled but retained consciousness. He has had no further fits.

In May, 1940, he developed a small swelling over the temporal bone just above the left ear. This was incised by a native doctor and is believed to have contained a quantity of pus.

In July, 1941, he first noticed another small swelling, this time in the left side of the neck over the upper third of the sternomastoid. Concurrently, he had pain in the left ear and his neck was stiff and painful on movement. He went to a European doctor and the condition rapidly improved with ear drops though the swelling remained. There was then a stationary period until early in 1942 when the swelling gradually increased in size. He was free from earache until June, 1942, when he was seized with a jabbing pain in the ear, associated with a high-pitched ringing noise. He states that his hearing was impaired during the attack.

In August, 1942, he had a further attack of earache which improved whilst he was being treated with a 3-day course of sulphapyridine. During the last 6 months he has had attacks when his vision has become misty and he has been unable to identify people clearly at 10 yards. These attacks have lasted only 1 or 2 minutes but they have been of frequent occurrence, as many as ten in a week, and usually take place in the early morning.

Since the operation on his neck in September, 1942, the patient has had no trouble with his eyes but occasionally feels light-headed and has recently noticed a tendency to fall over to the right side.

FIG. 1.—Pus. Operculated ova. × 320.

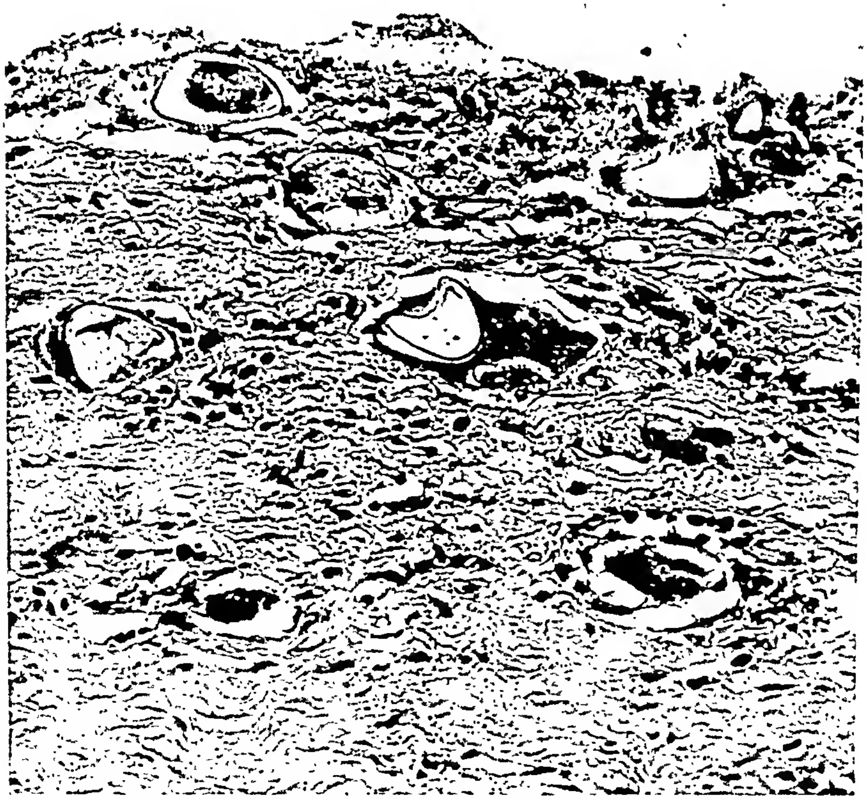

FIG. 2.—Cyst wall showing ova : one undergoing ingestion by foreign-body giant cell. × 270.

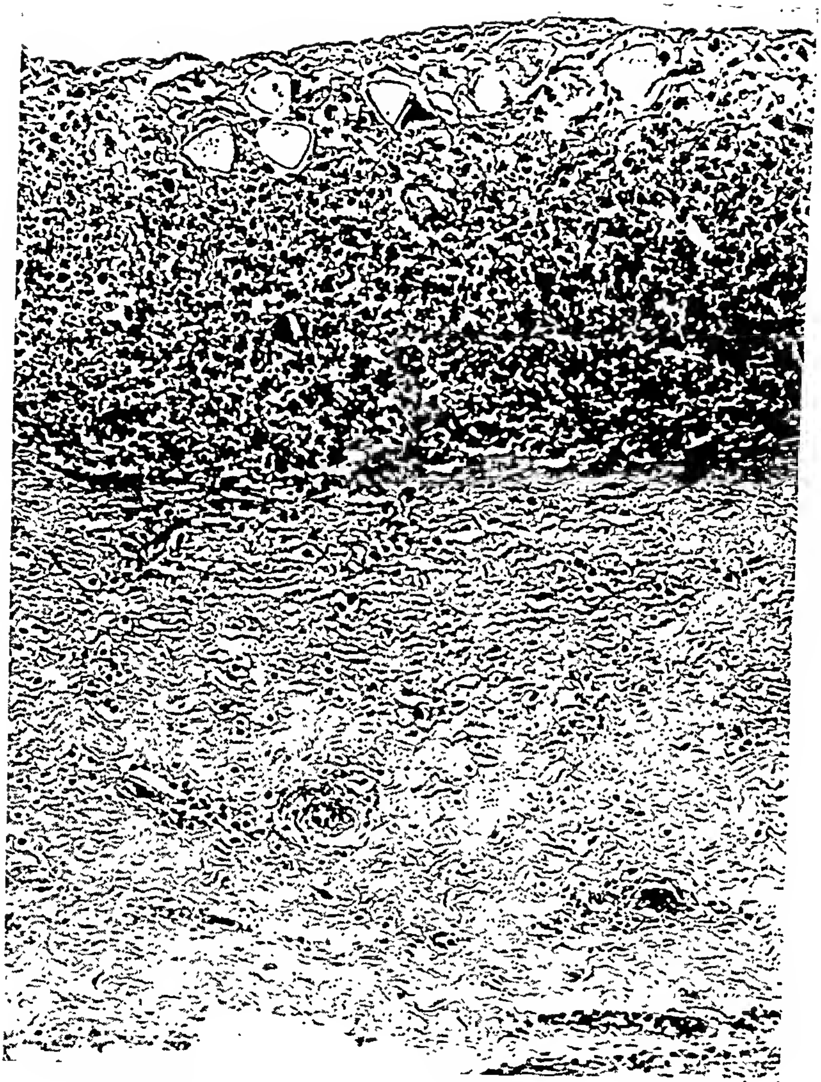

FIG. 3.—Cyst wall showing zonal demarcation. × 150.

Clinical Examination, November, 1942.

There were no signs or symptoms of any alimentary or respiratory disorder.

Radiological examination of the chest showed no abnormality.

The left external ear and meatus were normal. The drum showed no evidence of disease but there was considerable impairment of hearing, while bone conduction was greater than air conduction. The right ear was normal.

Central nervous system.

Right homonymous hemianopia and impaired vision of the whole right eye. The pupil reflexes and cranial nerves were all normal. The fundi were normal and there was no nystagmus.

Finger-nose test normal.

Slight Rombergism to the right side but no ataxia or dysdiadochokinesis.

In the arms and legs, the right side showed diminished muscular power and there was hypotonia of the left side. All deep reflexes of the left arm were diminished and the left knee jerk was obtainable only with reinforcement.

The ankle jerks were equal and normal. The plantar reflexes were normal. Radiological examination of the cranium revealed no abnormality.

Laboratory Examinations. September-November, 1942.

Pus from cyst in neck.

The pus had a homogeneous creamy consistency and was fawn-coloured. On microscopic examination it was found to contain numerous operculated ova indistinguishable from those of *Paragonimus.* They were broadly oval, pale yellowish-brown and showed no segmentation of the contents which were of a granular nature. There was no noticeable thickening of the shell at the opposite pole from the operculum, a feature usually described in the species *Paragonimus westermanii.* They measured $62\mu \times 40\mu$ after fixation of the pus in 10 per cent. formol-saline but, allowing for slight shrinkage, they were considerably smaller than the recorded sizes for *Paragonimus* species which are said to be from $70\text{-}90\mu \times 45\text{-}55\mu$. This is probably not an important point considering the natural variation in the size of ova in many helminths but, as the adult worm was not found in this case, it may possibly indicate a new species or variety in West Africa. The cells of the pus were mainly neutrophil polymorph leucocytes but nuclear degeneration was marked and many cells could not be identified. Eosinophils were very scanty. (Fig. 1.)

Cyst from neck.

A thin walled incomplete fibrous sac measuring 5×4 cm., and 1 or 2 mm. in thickness. Muscle fibres were blended with the outer surface and the inner surface was yellowish-brown in colour and finely granular in appearance. (Figs. 2 and 3.) A careful search failed to reveal any adult worms.

Histology.

Outer and inner zones could be distinguished and were fairly sharply demarcated from one another. The outer zone was of variable thickness and consisted of collagen fibres with an occasional bundle of striped muscle. It was well supplied with small blood vessels and sparsely infiltrated with inflammatory cells, mainly of the plasma cell type. An occasional foreign body giant cell and a few ova were present. The inner zone consisted of vascular granulation tissue intermingled with collagen fibres and richly infiltrated with ova and inflammatory cells. Many of the ova were distorted and attempts at phagocytosis by foreign-body giant cells were evident. Eosinophils were scanty and most of the inflammatory cells were plasma cells.

There was considerable haemorrhage into the granulation tissue. The depth of this zone was variable and in some cases granulation tissue was replaced by the collagenous fibrous tissue of the outer zone.

Blood examination :

Differential counts were carried out on two occasions, before and after removal of the cyst. The figures were : Polymorphs 29 per cent., lymphocytes 61 per cent., monocytes 2 per cent., eosinophils 8 per cent.

It may be noted that high lymphocyte counts are of common occurrence in Nigeria and in many cases it is impossible to assess their significance.

Kahn test :

Positive (++ reaction) 3rd November, 1942.

The cerebrospinal fluid would also have been examined but the patient, a soldier, was no longer available. He came from a district in which yaws is endemic and acknowledged having had the disease as a child. He denied having had syphilis.

Faeces examination : Ova of *Ascaris lumbricoides.*

Sputum examination after enforced coughing: Nothing abnormal found.

Comment.

It is difficult to see how the physical signs can fit into any single lesion of the central nervous system. The history of a fit 11 years ago and the present-day findings of an homonymous hemianopia with normal pupil reflexes suggest there is a lesion of the left cerebrum posterior to the corpora quadrigemina. The hypotonia and depressed knee jerks on the left side may be explained by a left cerebellar or vestibular lesion. In addition there is the history of a previous swelling around the left ear and the present evidence of a *Paragonimus* cyst of the neck associated with middle ear deafness. It is suggested that this

is a case in which the flukes have given rise to multiple lesions of the central nervous system and have also migrated to the region of the left ear.

While paragonimiasis is usually associated with lung disease no region of the body is immune from the migrations of the parasites and ill effects from their presence may arise anywhere.

We assume that this is a case of *Paragonimus* infection as the only ova of similar character found in man are those of *Dibothriocephalus latus*, a parasite which is confined to the intestine.

Summary.

A case of a cyst in the left side of the neck containing *Paragonimus* ova is described in a West African native. It was associated with signs and symptoms of lesions in the central nervous system and the left ear, and it is suggested that these may also be due to *Paragonimus* infection.

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PRELIMINARY REPORT ON PENTAMIDINE IN THE TREATMENT OF LATE CASES OF SLEEPING SICKNESS.

BY

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Colonial Medical Service, Northern Rhodesia.

Up to date fourteen cases have been treated with pentamidine (M. & B. 800). All these patients were Africans and they all complained of drowsiness, and were definitely cases of trypanosomiasis, though trypanosomes have not invariably been found. Trypanosomes were found at one time or another in blood, glands or cerebrospinal fluid of Cases 1, 2, 5, 8, 9, 10, 11 and 14.

The drug was tested out by the intramuscular route to start with, dosage varying between 1·6 and 5·15 mg. per kg. of body weight, this dose being dissolved in 1 c.c. of water. In no case of intramuscular injection has there been any reaction to the drug, nor any pain or discomfort at the site of the injection. A daily injection has been given in each case for 8 consecutive days. Intravenously a dose of more than 2·0 mg. per kg. was found to produce headaches and severe rigors.

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These cases were all *gambiense* infections, there being no *rhodesiense* cases in this neighbourhood of Northern Rhodesia; and all, except Case 5, have been infected while living on the shores of Lake Tanganyika where *Glossina palpalis* is plentiful. Case 5 has been resident for some years at Kasenga on the Belgian Congo side of the Luapula River.

As trypanosomes are infrequently found in these cases, and these disappear after treatment, the only way of assessing the immediate results of treatment is by repeated lumbar puncture and a cell count of the cerebrospinal fluid.

Notes of the Fourteen Cases.

Case 1.

Female aged 45. Old case, first treated for trypanosomiasis in December, 1940. Since then had been well until 2 weeks before admission when she became drowsy.

6.7.42.—Admitted to hospital, moderately drowsy. Blood positive for trypanosomes; formol-gel positive; weight 90 lb.; C.S.F. 1,500 cells per cu. mm. No trypanosomes found in C.S.F.

18.7.42.—*Treatment*: Pentamidine 1 grain daily given intramuscularly for 8 consecutive days (1·6 mg. per kg. body weight).

5.8.42.—C.S.F. 60 cells per cu. mm. Condition improved.

20.8.42.—C.S.F.—failed to obtain any. Condition much improved, no drowsiness.

4.9.42.—Discharged; patient unwilling to stay in hospital any longer. Clinically quite normal. Weight 95 lb.

12.10.42.—Seen at her village; now not at all well; very weak and is partially paralysed; refuses to come to hospital.

27.12.42.—Reported to have died at her village.

Case 2.

Male aged 15. Old case treated in hospital here in 1940, and in December, 1941, with antrypol and tryparsamide. Drowsiness commenced again 1 week before admission.

29.6.42.—Admitted to hospital; weight 102 lb.; blood negative now but was positive in December, 1941. Formol-gel positive; C.S.F. 200 cells per cu. mm.

1.7.42.—*Treatment*: 2 grammes tryparsamide, and also on 4th, 8th and 15th July.

20.7.42.—Daily injections of 1 grain pentamidine intramuscularly for 8 days (1·4 mg. per kg.).

5.8.42.—C.S.F. 190 cells per cu. mm.

12.8.42.—C.S.F. 120 cells per cu. mm. No drowsiness now.

20.8.42.—C.S.F. 30 cells per cu. mm. Is apparently quite well.

4.9.42.—Discharged, clinically normal.

Case 3.

Male aged 25. New case, complains of drowsiness of about 1 year's duration, and itchiness of skin of 1 week's duration.

18.6.42.—Admitted. Very dull mentally, continually dozing, blood negative, gland puncture negative, formol-gel positive, weight 161 lb.

27.6.42.—*Treatment*: Tryparsamide 2 grammes, repeated on 1st July and 4th July.

10.7.42.—Dimness of vision complained of.

25.7.42.—Pentamidine 2 grains (1·9 mg. per kg.) intramuscularly daily for 8 days.

5.8.42.—C.S.F. 80 cells per cu. mm. No obvious improvement in drowsiness.

12.8.42.—C.S.F. blood stained.

20.8.42.—C.S.F. 60 cells per cu. mm.

27.8.42.—C.S.F. 40 cells per cu. mm. Vast improvement in mental condition—no drowsiness.

3.9.42.—C.S.F. 10 cells per cu. mm.

4.9.42.—Discharged. Perfectly normal clinically. Weight 158 lb.

Case 4.

Male aged 35. New case ; complains of headaches and drowsiness of 3 weeks' duration.

27.7.42.—Admitted. Blood negative. Gland puncture negative. Formol-gel positive. C.S.F. 1,180 cells per cu. mm. No trypanosomes found in C.S.F.

29.7.42.—*Treatment* : Pentamidine 2 grains daily intramuscularly (2·27 mg. per kg.) for 8 days.

12.8.42.—C.S.F. 780 cells per cu. mm.

20.8.42.—C.S.F. failed to obtain any fluid.

23.8.42.—Discharged. Clinically very fit. Weight 130 lb. Would not stay in hospital any longer.

Case 5.

Male aged 25 to 30. New case ; has been resident for 3 years at Kasenga, Belgian Congo, where he said he was bitten by tsetse (whether *G. morsitans* or *G. palpalis* occurs there I do not know).

3.8.42.—Admitted, complaining of drowsiness of 3 months' duration, with pain in left side of body and a twitching big toe. Mentally he is very dull and resembles parkinsonism in appearance. Blood negative. Gland puncture negative. Formol-gel test positive. C.S.F. 330 cells per cu. mm. No trypanosomes in C.S.F. Weight 132 lb.

6.8.42.—*Treatment* : Pentamidine 2 grains (2·2 mg. per kg.) daily, intramuscularly for 8 consecutive days.

20.8.42.—C.S.F. 320 cells per cu. mm.

27.8.42.—C.S.F. blood stained.

29.9.42.—C.S.F. 130 cells per cu. mm. Patient is very weak and drowsy. Trypanosomes found in C.S.F.

30.9.42.—Intravenous pentamidine, 1 grain daily for 8 days.

5.10.42.—Pentamidine appears to be doing no good—more drowsy than ever, and practically comatose. Given tryparsamide, 2 grammes intravenously, at 4-day intervals, for three injections. Very rapid improvement.

19.11.42.—C.S.F. 160 cells per cu. mm.

10.12.42.—C.S.F. 80 cells per cu. mm. Patient is much brighter and up and about.

Case 6.

Male aged 12. New case. Complains of drowsiness of 1 month's duration.

10.8.42.—Admitted. Dull mentally, and very drowsy. Blood negative for trypanosomes. Positive malaria. C.S.F. 400 cells per cu. mm. No trypanosomes found. Malaria treated first. Weight 57 lb.

18.8.42.—*Treatment*: Pentamidine 2 grains (5·15 mg. per kg.) daily, intramuscularly for 8 days. This was an experimentally large dose.

23.8.42.—Faint trace of albumin in urine, and a few granular casts. No complaint of any discomfort or feeling unwell by patient; 8 days' course of treatment completed with no other signs or symptoms.

3.9.42.—C.S.F. blood stained. As this had to be done under a general anaesthetic, each time, it was decided to discharge him and examine him again in 2 months' time.

3.9.42.—Discharged—clinically very fit.

Case 7.

Male aged 16. Old case—was treated in Abercorn, 2 years ago.

18.8.42.—Admitted. Now complains of drowsiness of some months' duration. On examination is drowsy. Blood negative. C.S.F. 520 cells per cu. mm. Weight 125 lb.

21.8.42.—*Treatment*: Pentamidine 3 grains (3·52 mg. per kg.) daily, intramuscularly, for 8 days. No untoward signs or symptoms during treatment.

3.9.42.—C.S.F. 20 cells per cu. mm.

7.9.42.—Discharged—clinically very well.

Case 8.

Male aged 14. Old case, first treated in January, 1941, and again in October, 1941, with both antrypol and tryparsamide.

14.9.42.—Admitted. Now complains of drowsiness of 3 weeks' duration; is very drowsy. Blood, positive trypanosomiasis. C.S.F. 300 cells per cu. mm. Weight 90 lb.

15.9.42.—*Treatment*: Pentamidine 2 grains (3·26 mg. per kg.) daily, for 8 days. Has been pyrexial during whole course of treatment, and complained of headache.

29.9.42.—C.S.F. 200 cells per cu. mm. No trypanosomes. Still very drowsy.

30.9.42.—Intravenous pentamidine, 1 grain (1·63 mg. per kg.) for 8 days. Temperature now normal, but still very drowsy.

9.10.42.—Tryparsamide 1 gramme, intravenously, given as he appeared to be going into a coma.

13.10.42.—Tryparsamide 1 gramme, intravenously.

14.10.42.—Patient appears much brighter, and is up and about.

19.11.42.—C.S.F. 130 cells per cu. mm. Vast improvement mentally.

10.12.42.—C.S.F. 60 cells per cu. mm. Improvement maintained.

Case 9.

Male aged 12. Old case—first treated for trypanosomiasis in January, 1940, with antrypol and tryparsamide.

17.9.42.—Readmitted complaining of drowsiness. Mentally unstable. No enlarged glands. Positive Kerandel sign. Positive formol-gel. Negative blood. C.S.F. 230 cells per cu. mm. Trypanosomes in C.S.F.

22.9.42.—*Treatment*: Pentamidine 2 grains (3·4 mg. per kg.) daily, intramuscularly, for 8 days.

12.10.42.—C.S.F.—failed to obtain any, as patient was too obstreperous.

28.10.42.—C.S.F. 10 cells per cu. mm. Is mentally alert and physically much improved.

30.10.42.—Discharged.

13.11.42.—Seen at his village—very fit mentally and physically.

Case 10.

Male aged 14. Old case—first treated for trypanosomiasis in June, 1941. Positive blood and gland fluid then.

10.10.42.—Readmitted, complaining of occasional drowsiness, otherwise appears fit and normal. Blood negative. C.S.F. 150 cells per cu. mm. No trypanosomes.

14.10.42.—*Treatment*: Pentamidine 2 grains (3.3 mg. per kg.) intravenously.

15.10.42.—Pentamidine 2 grains intravenously. Complains of headache and rigors after above dosage.

16.10.42.—Pentamidine 1 grain (1·65 mg. per kg.) intravenously given for next 6 days without any ill effects.

28.10.42.—C.S.F. 30 cells per cu. mm. No longer drowsy. Looks and feels much better. Discharged.

12.11.42.—Seen at his village. Appears very bright and in excellent health.

27.11.42.—Seen at his village. Reports that he is very well. Looks very fit.

Case 11.

Female aged 25. Old case—first treated for trypanosomiasis in 1936. Readmitted in 1940, and in August, 1941. Positive trypanosomes. Had antrypol and tryparsamide.

10.10.42.—Readmitted. Very drowsy—can't keep awake for half an hour at a time; very thin; mentally dull. No other signs or symptoms. Blood negative. C.S.F. 100 cells per cu.mm. No trypanosomes found.

14.10.42.—*Treatment*: Pentamidine 2 grains (3·3 mg. per kg.) intravenously.

15.10.42.—Pentamidine 2 grains (3·3 mg. per kg.) intravenously. Complains of headache and rigors.

16.10.42.—Dosage reduced to 1 grain for next six injections.

28.10.42.—C.S.F. 70 cells per cu. mm.

7.11.42.—Now no longer drowsy. Looks much better and feels fit. Discharged.

13.11.42.—Seen at village—appears quite fit and has no drowsiness.

Case 12.

Female aged 45. Old case—first treated for trypanosomiasis in 1940.

14.10.42.—Readmitted, complaining of slight drowsiness. Blood negative. C.S.F. 180 cells per cu. mm. Negative for trypanosomes.

29.10.42.—*Treatment*: Pentamidine 1 grain (1·44 mg. per kg.) intravenously, daily for 8 days.

19.11.42.—C.S.F. 60 cells per cu. mm. Feels very fit and is no longer drowsy.

12.12.42.—Discharged. Very fit.

Case 13.

Male aged 25. Old case—first treated for sleeping sickness in January, 1940.

22.11.42.—Readmitted. Is unable to walk. Drowsing all day. Is unable to cerebrate or answer questions. Incontinent. Blood is negative. C.S.F. under pressure, 430 cells per cu. mm. No trypanosomes found.

26.11.42.—*Treatment*: Pentamidine 1 grain (1·5 mg. per kg.) intravenously, daily for 8 days.

5.12.42.—No improvement. Is semi-comatose.

6.12.42.—Tryparsamide 2 grammes, intravenously.

8.12.42.—Is going into coma.

10.12.42.—Died in coma. No trypanosomes found in blood.

Case 14.

Male aged 14. Old case—first treated in July, 1941, when he had trypanosomes in his blood.

13.12.42.—Readmitted complaining of drowsiness. No enlarged glands. No rash, but has very itchy skin. Blood positive trypanosomes. C.S.F. 270 cells per cu. mm.—increased pressure. No trypanosomes found.

18.12.42.—*Treatment*: Intravenous pentamidine 1 grain (1·5 mg. per kg.) daily for 8 days.

30.12.42.—Patient seems much brighter in himself.

CONCLUSIONS.

In late cases of *T. gambiense* sleeping sickness pentamidine certainly reduces the cell count of the cerebrospinal fluid; in cases where the disease is not too far advanced there is a very real improvement in the clinical condition, though a follow-up period of at least 2 years is necessary to establish its real value. Advanced cases, verging on coma, do not appear to derive any benefit; while in Cases 5 and 8 tryparsamide produced a rapid improvement clinically, after pentamidine had failed. It is considered that doses of at least 2·0 mg. per kg. of body weight should be given, and that the intravenous route is better than the intramuscular. Probably treatment carried out for a longer period than 8 days might be more effective. No ill effects follow the dosage of 5 mg. per kg. intramuscularly, given daily for 8 days.

It is considered that the drug is worthy of a further trial in the dosage mentioned above, and possibly two courses of eight injections, intravenously, with a week's interval in between, will prove highly effective—except in cases verging on the stage of coma.

TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
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MYOSITIS TROPICA.

BY

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AND

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Capt., R.A.M.C., Pathologist, and Research Fellow in Haematology, Christie Hospital and Holt Radium Institute, Manchester.

This paper describes the clinical features of tropical myositis and adds a note on the pathology. Our observations are based on a series of twenty African patients, natives of Nigeria (eleven cases), the Cameroons (seven cases), and the Gold Coast (two cases), who were admitted with this disease into the Surgical Division of a West African Military Hospital. They constitute approximately 1 per cent. of all African admissions to the hospital.

CLINICAL FEATURES.

The patients were admitted with a moderate pyrexia and a tender swelling, diffuse or circumscribed, involving as a rule the muscles of either the upper or the lower limb. Occasionally two such swellings were present or a second one developed while the patient was in hospital. These lesions represented either acute abscesses (pyomyositis), or areas of focal necrosis, and in several instances it was difficult to distinguish between the two on clinical grounds. Some unnecessary incisions were made, since even those swellings that exhibited acute tenderness, heat and fluctuation were occasionally found to reveal no

* We wish to express our thanks to Col. J. P. J. JENKINS, A.M.S., for permission to publish this communication.

TABLE.

Case Number	Race.	Duration of symptoms (days) before admission.	Site of Swelling.	Operation.
1	Nigeria	14	Adductor muscles, left thigh	Incision, no pus, biopsy
2	Cameroons	4	Flexors forearm, right (a)	Incision, pus, biopsy
			Pectoralis major, left (a)	None
3	Cameroons	11	Quadriceps, left	Incision, pus
4	Nigeria	?	Hamstrings, left	Incision, no pus
5	Cameroons	14	Below left scapula	Incision, pus
6	Nigeria	3	Peroneal region, left	None
7	Nigeria	20	Quadriceps, left	None
8	Nigeria	3	Quadriceps, left	None
9	Nigeria	10	Quadriceps, right	Incision, pus
10	Gold Coast	14	Anterior abdominal wall	None
11	Gold Coast	7	Forearm, right	None
12	Nigeria	2	Lower left six ribs, posterior (b)	Incision, pus
			Pectoralis major, left (c)	None
13	Cameroons	7	Tensor F. femoris, right	None (d)
			Flexors forearm, left	None
14	Cameroons	14	Outer side elbow, left	None
15	Nigeria	2	Forearm, right	None
16	Nigeria	5	Calf muscles, right	None
			Quadriceps, right	None
17	Cameroons	8	Quadriceps, right	None
			Hamstrings, left	None
18	Nigeria	7	Hamstrings, left	Incision, no pus, biopsy
			Scarpa's triangle, left (e)	None
19	Nigeria	7	Flexors forearm, right	None
			Scapula region, right	Incision, no pus
20	Cameroons	3	Pectoralis major, right	None.

(a) Developed 11 days after admission.
(b) Had been struck over the back with a stick 1 week before onset of symptoms.
(c) Developed 7 days after admission.
(d) Swellings subsided spontaneously and he was discharged from hospital after 14 days. Ten days later he was re-admitted with an abscess in the tensor F. femoris, which was drained.
(e) Developed 16 days after admission.

more than a glassy oedematous condition of the muscle. The regional lymphatic glands were rarely affected. In those cases that did not proceed to suppuration the symptoms might continue for a week or more before abating, and in our experience the administration of sulphapyridine was without effect. Subsequent contractures of the affected muscles were not a feature of the lesion. Case 2 alone showed a slight temporary contracture of the flexors of the middle finger.

Case 18.

A typical case. The man, age 25, was admitted to hospital on 19th October, 1942, with a circumscribed tender swelling about 5 by 4 inches, spontaneous in origin and involving the left hamstring muscles in their upper half. Symptoms were of 1 week's duration. Temperature, 101·6° F. Two days later there was no improvement in his condition and he was put on to sulphapyridine by mouth. By 24th October he had received 18 grammes, but the evening temperature was up to 103° F. and there was no change in the character of the swelling. Kahn precipitation test was negative, and the white blood cell count was 11,000 per c.mm. Red blood cells showed no sickling *in vitro*. On 26th October the man was still complaining of great pain and tenderness in the thigh, and an incision was made over the swelling. This revealed nothing but an indefinite oedema of the muscle, a portion of which was excised for histological investigation. Subsequently pyrexia and pain gradually subsided. On 4th November a tender lump developed which involved the muscles in Scarpa's triangle on the left side, but this resolved spontaneously within a few days and gave rise to no constitutional disturbance.

In those cases in which suppuration had occurred incision revealed an acute abscess cavity containing thick yellowish-white pus from which *Staphylococcus aureus* was isolated in the few instances in which the pus was cultured.

Biopsy Reports.

Case 1.

The muscle tissue has undergone degenerative changes of the coagulative type, with complete loss of striations, areas of haemorrhage between the muscle fibres and a monocytic infiltration of the inter-fibrillar connective tissue. Much fibroblastic reaction is present in completely necrotic areas.

Case 2.

There is much haemorrhage into the muscle tissue spaces, accompanied by a mononuclear cell infiltration. The muscle fibres are degenerate and in parts completely necrotic. There is a surrounding fibroblastic reaction.

Case 18.

There is much degeneration of the voluntary muscle fibres of the coagulative type, the sarcolemma having completely disappeared and a syncytial mass of necrotic muscle tissue remaining. This is accompanied by areas of haemorrhage into the interstitial tissue, a monocytic infiltration and surrounding fibroblastic reaction.

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Commentary.

We consider that tropical myositis is primarily an acute degenerative condition, characterized by haemorrhage into the intermuscular tissue spaces together with a mononuclear cell infiltration, producing an appearance similar to the coagulative necrosis of muscle (Zenker's degeneration), that has been described in typhoid fever, influenza, tetanus, etc. Resolution occurs by fibrosis unless secondary infection intervenes and leads to suppuration (pyomyositis).

We have not studied the aetiological factors in detail, but the relationship of the condition to trauma, helminthic infestations, syphilis, yaws and the sickle cell trait appears to be very uncertain. In no instance have we observed a collection of the so-called "serous" fluid described by Scott (1912), and Manson-Bahr (1940).

References.

Manson-Bahr, P. (1940). *Manson's Tropical Diseases*, p. 705, 11th ed. London: Cassell & Co.

Scott, H. H. (1912). *J. trop. Med. Hyg.*, 15, 97.

CORRESPONDENCE.

FIELD'S STAIN.

To the Editor, TRANSACTIONS *of the Royal Society of Tropical Medicine and Hygiene.*

SIR,

We have used for some months a modification of Field's* quick method of staining thick blood films.

Preliminary experiments were made some time ago in order to find out which chemical in the eosin group of stains, and which in the methylene-azur compound will give the best Romanowsky effect in aqueous solution.

It was found that erythrosin gives a quicker and clearer staining than eosin, and of the various methylene blue solutions at our disposal, Unna's polychrome methylene gave the best results.†

Field's quick method of staining was used in this laboratory for several months with good results. The conservation of the form of the malaria parasite proved to be a great aid in microscopical diagnosis.

But quite often we could not obtain with Field's method a Romanowsky effect with purple staining of the chromatin. Another drawback was the fact that spirochaetes of relapsing fever were very often understained and almost invisible.

An attempt was made, therefore, to substitute eosin by erythrosin and azur I with methylene blue by Unna's polychrome methylene blue.

The composition of the stains is therefore as follows :—

Solution A.	*Grammes*
Unna's polychrome methylene blue	5·0
Disodium hydrogen phosphate (anhydrous) ...	5·0
Potassium dihydrogen phosphate (anhydrous)	6·25
Distilled water	500·0

Solution B.	*Grammes*
Erythrosin	1·0
Disodium hydrogen phosphate (anhydrous) ...	5·0
Potassium dihydrogen phosphate (anhydrous)	6·25
Distilled water	500·0

* FIELD, J. W. (1941). Further note on a method of staining malarial parasites in thick blood films. *Trans. R. Soc. trop. Med. Hyg.*, 35, 35.

† All the stains used were manufactured by Carlo Erba, Milan.

These solutions should be filtered and are then ready for immediate use.

The technique of staining is: A blood drop, not too thick, freshly dried :—

1. Stained for 1 second in the A solution.
2. Rinsed in tap water for 2 to 3 seconds.
3. Stained for 1 second in B solution, and
4. Rinsed again and dried.

This modified staining method has proved to be very satisfactory in our hands. The time of staining is about 5 seconds, and the Romanowsky effect with purple staining of the chromatin, as well as a clear blue staining of spirochaetes, when present, is invariably obtained.

I am, etc.,

M. WOLMAN,
Capt., R.A.M.C.

Menelik Hospital,
Addis Ababa, Ethiopia.

A SIMPLE DEVICE FOR DESTROYING ADULT MOSQUITOES, HOUSE-FLIES AND OTHER HOUSEHOLD PESTS BY THE USE OF A FLAME THROWER.

To the Editor, TRANSACTIONS *of the Royal Society of Tropical Medicine and Hygiene.*

SIR,

A flame thrower has been used very successfully by the writer for destroying mosquitoes in Cyprus since May, 1920. Its use is confined to places such as stables, out-houses, caves, cellars, sheepfolds, air-raid shelters and other situations where there is no fear of fire or damage being caused.

In the beginning several devices were tried with varying success, but the best was found to be an ordinary " flit-gun" fitted with a small burner so as to ignite the spray. The air pressure throws out a tongue of flame 1½ to 2 feet long and about 6 to 8 inches wide at each stroke.

The small flame, necessary for igniting the spray, is obtained by having a small tube of about ¼-inch bore (wick-holder), 1¼ inch high and about 2 to 2½ inches in front of the spray nozzle. The wick-holder is soldered on the container of the flit-gun and has an ordinary wick which obtains its fuel from the container. In addition to the usual petroleum insecticides for general purposes, ordinary light fuel oil was tried with satisfactory results.

Other advantages of this small burner are that it serves as a lamp, producing sufficient light for the operator to see his way while going through dark places and the smoke from the flame dislodges the insects from unobserved places and exposes them to destruction by the spray or flame.

The flame thrower is also useful for the destruction of sand-flies, house-flies, cockroaches, bed-bugs, ants, moths and fleas, as well as the eggs and larvae of the majority of them.

The flame often destroys the insects outright and those which survive generally have the legs or wings so scorched as to render them harmless.

By extinguishing the small burner in front of the spray nozzle the flit-gun can be used in the usual way for spraying insecticides.

I have to thank the Malariologist of the Middle East Command for the suggestion that this apparatus should be made more widely known and the Acting Director of Medical Services of Cyprus for permission to publish it.

I am, etc.,

M. Aziz,
Chief Sanitary Inspector, Cyprus.

Nicosia,
Cyprus.

CORRIGENDUM.

Vol. XXXVI. No. 5. Paper by Reitler & Marberg.

Heading at top of alternate pages 306-318 should read : Tin in Treatment of Typhoid (instead of Tin in Treatment of Typhus).

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